MycoKeys

MycoKeys 100: 123–151 (2023)
DOI: 10.3897/mycokeys.100.109423

Research Article

Taxonomic and phylogenetic characterisations of six species of Pleosporales (in Didymosphaeriaceae, Roussoellaceae and Nigrogranaceae) from China

Hongmin Hu[1,2*], Minghui He[1,2*], Youpeng Wu[1], Sihan Long[1], Xu Zhang[1], Lili Liu[3], Xiangchun Shen[1,2], Nalin N. Wijayawardene[4,5], Zebin Meng[6], Qingde Long[1], Jichuan Kang[7], Qirui Li[1,2]

1 *State Key Laboratory of Functions and Applications of Medicinal Plants, Guizhou Medical University, Guiyang, Guizhou province, China*
2 *The High Efficacy Application of Natural Medicinal Resources Engineering Center of Guizhou Province (The Key Laboratory of Optimal Utilization of Natural Medicine Resources), School of Pharmaceutical Sciences, Guizhou Medical University, University Town, Guian New District, Guiyang, Guizhou province, China*
3 *Key Laboratory of Infectious Immune and Antibody Engineering of Guizhou Province, Cellular Immunotherapy Engineering Research Center of Guizhou Province, Immune Cells and Antibody Engineering Research Center of Guizhou Province, School of Biology and Engineering, Guizhou Medical University, Guiyang, Guizhou province, China*
4 *Center for Yunnan Plateau Biological Resources Protection and Utilization, College of Biological Resource and Food Engineering, Qujing Normal University, Qujing, Yunnan province, China*
5 *Tropical Microbiology Research Foundation, 96/N/10, Meemanagoda Road, 10230 Pannipitiya, Sri Lanka*
6 *Guizhou Tea Seed Resource Utilization Engineering Research Center, Guizhou Education University, Guiyang, Guizhou province, China*
7 *The Engineering and Research Center for Southwest Bio-Pharmaceutical Resources of National Education Ministry of China, Guizhou University, Guiyang, Guizhou province, China*

Corresponding authors: Qirui Li (lqrnd2008@163.com); Qingde Long (longqingde@gmc.edu.cn)

OPEN ACCESS

Academic editor: J. Luangsa-ard
Received: 12 July 2023
Accepted: 27 October 2023
Published: 29 November 2023

Citation: Hu H, He M, Wu Y, Long S, Zhang X, Liu L, Shen X, Wijayawardene NN, Meng Z, Long Q, Kang J, Li Q (2023) Taxonomic and phylogenetic characterisations of six species of Pleosporales (in Didymosphaeriaceae, Roussoellaceae and Nigrogranaceae) from China. MycoKeys 100: 123–151. https://doi.org/10.3897/mycokeys.100.109423

Copyright: © Hongmin Hu et al.
This is an open access article distributed under terms of the Creative Commons Attribution License (Attribution 4.0 International – CC BY 4.0).

Abstract

Pleosporales comprise a diverse group of fungi with a global distribution and significant ecological importance. A survey on Pleosporales (in Didymosphaeriaceae, Roussoellaceae and Nigrogranaceae) in Guizhou Province, China, was conducted. Specimens were identified, based on morphological characteristics and phylogenetic analyses using a dataset composed of ITS, LSU, SSU, *tef*1 and *rpb*2 loci. Maximum Likelihood (ML) and Bayesian analyses were performed. As a result, three new species (*Neokalmusia karka*, *Nigrograna schinifolium* and *N. trachycarpus*) have been discovered, along with two new records for China (*Roussoella neopustulans* and *R. doimaesalongensis*) and a known species (*Roussoella pseudohysterioides*). Morphologically similar species and phylogenetically close taxa are compared and discussed. This study provides detailed information and descriptions of all newly-identified taxa.

Key words: phylogeny, saprophytic fungi, taxonomy, three new taxa

Introduction

The order Pleosporales was formally established by Luttrell and Barr (1987) and is characterised by perithecioid ascomata with a papillate apex, ostioles with or without periphyses, cellular pseudoparaphyses, bitunicate asci and ascospores of varying shapes, pigmentation and septation (Zhang et al. 2012).

* These authors contributed equally as co-first authors.

As one of the largest orders in the Dothideomycetes, it comprises a quarter of all dothideomycetous species (Ahmed et al. 2014b). Species in this order are found in various habitats and can be epiphytes, endophytes or parasites of living leaves or stems, hyperparasites on fungi or insects, lichenised or saprobes of dead plant stems, leaves or bark (Ramesh 2003; Kruys et al. 2006). In this study, we identified six species belonging to the order Pleosporales from the families Didymosphaeriaceae Munk, Nigrogranaceae Jaklitsch & Voglmayr and Roussoellaceae Jian et al. in Guizhou, China (Wijayawardene et al. 2022).

The family Didymosphaeriaceae, introduced by Munk (1953) and typified by *Didymosphaeria fuckeliana*, can be placed in the order Pleosporales. *Neokalmusia* was introduced to Didymosphaeriaceae by Ariyawansa et al. (2014a). Currently, only eight *Neokalmusia* species are listed in Index Fungorum (accession date: 25 July 2023). Members of Didymosphaeriaceae are known to form numerous different types of life modes, including saprobes, pathogens or endophytes and can be found both on land and in water (Gonçalves et al. 2019; Hongsanan et al. 2020). In the study of this paper, *Neokalmusia karka* is taken from the dead culms of the *Phragmites karka* (Retz.) Trin. ex Steud. Shilihe Beach Park, Huaxi, Guizhou Province, China.

Roussoellaceae was established to accommodate three genera, *Neoroussoella* Jian K. Liu et al., *Roussoella* Sacc. and *Roussoellopsis* I. Hino & Katum., based on molecular phylogenetic studies (Liu et al. 2014). The genus *Roussoella* has cylindrical asci with *Cytoplea* asexual morphs, which distinguishes it from other genera (Liu et al. 2014). Another feature reported for the genus *Roussoella* is the high stability of the ascal exotunica, particularly in 3% potassium hydroxide (KOH). This is quite common for nearly all fungi treated here, while only in *Nigrograna* can fissitunicate ascus dehiscence be seen rather frequently (Jaklitsch and Voglmayr 2016). Nigrogranaceae was established to accommodate *Nigrograna*, with *N. mackinnonii* (Borelli) Gruyter et al. as the type species (Jaklitsch and Voglmayr 2016). As the only genus in the family Nigrogranaceae, *Nigrograna* was established despite lacking strong bootstrap values support in ITS/*tef*1-based phylogenetic trees (Kolařík et al. 2017; Mapook et al. 2020; Zhang et al. 2020a; Wijayawardene et al. 2020). Species of *Nigrograna* may be interpreted as a result of cryptic speciation, as, morphologically, they show only subtle differences (Jaklitsch and Voglmayr 2016). Twenty-three *Nigrograna* species are listed in Index Fungorum (accession date: 25 July 2023).

In this study, we collected dead branches in Guizhou Province, China. Examination of the wood revealed three novel fungal species, two species that are newly recorded in China and one known species of Pleosporales. To elucidate their taxonomic placement and relationships with related species, we conducted morphological observations and phylogenetic analyses, based on combined ITS, LSU, SSU, *tef*1, and *rpb*2 sequences. Detailed descriptions of the morphological features of these species along with their molecular characterisation are provided.

Materials and methods

Fungal sampling, isolating and morphology

Fresh fungal specimens were collected in Duyun, Zunyi, Qiannan Prefecture and Guiyang, Guizhou Province and were brought back to the laboratory in self-seal-

ing bags. The specimens were then examined for their macroscopic characteristics using a Nikon SMZ 745 series stereomicroscope and photographed, using a Canon 700D digital camera. Micro-morphological structures were photographed using a Nikon digital camera (Canon 700D) that was attached to a light microscope (Nikon Ni). Melzer's iodine reagent was used to test the apical apparatus structures for amyloid reaction. Measurements of the specimens were registered using Tarosoft (R) Image FrameWork 80 software. The photo plates were arranged and improved using Adobe Photoshop CS6 software. Pure cultures were obtained with the single spore isolation method (Long et al. 2019) and the cultures were grown on potato dextrose agar (PDA) for preservation and observation of the anamorph (Rogers and Ju 1996). The specimens were deposited in the Herbaria of Guizhou Medical University (**GMB**) and Kunming Institute of Botany, Chinese Academy of Sciences (**KUN-HKAS**). Living cultures were deposited at the Guizhou Medical University Culture Collection (**GMBC**).

DNA extraction, polymerase chain reaction (PCR) amplification

The pure cultures were cultivated on potato dextrose agar (PDA) medium (Weigh 40.1g of potato dextrose agar (Shanghai Bowei Microbial Technology Co., Ltd.), add 1L of sterile water, and dissolve by heating until boiling. After dissolution, distribute the solution into conical flasks and place them in a high-pressure sterilizer for sterilization. Sterilization conditions are set at 121 degrees Celsius for 30 minutes. After sterilization, add a small amount of injectable potassium penicillin (Huamu) and injectable streptomycin sulfate (Huamu) into the culture medium and mix well. Pour the mixture into disposable culture dishes for later use. This step should be performed in aseptic conditions inside a laminar flow hood.) at 25 °C in the dark for 15–20 days. Fresh mycelium was collected by scraping it with a surgical knife and then transferred to a 1.5 ml centrifuge tube. DNA extraction was performed according to the instructions provided in the Biospin Fungus Genomic DNA Extraction Kit (BIOMIGA®).

The amplification of internal transcribed spacers (ITS), small subunit rDNA (SSU), large subunit rDNA (LSU), translation elongation factor 1-gene region (*tef*1) and RNA polymerase II second largest subunit (*rpb*2) was achieved using ITS5/ITS4, NS1/NS4, LR0R/LR5, EF1-938F/EF1-2218R and fRPB2-5f/fRPB2-7cr primers (Tibpromma et al. 2018; Vu et al. 2019; Wijesinghe et al. 2020; Dissanayake et al. 2021). The polymerase chain reaction (PCR) for the amplification of ITS, SSU, LSU, *tef*1 and *rpb*2 loci were performed using the Eppendorf Mastercycler nexus (SimpliAmp Thermal Cycler, A24811, SimpliAmp, China) gradient under the conditions specified in Table 1. Subsequently, the PCR fragments were sent to Sangon Biotech (Shanghai) Co., China, for sequencing. Amplification conditions using the Polymerase Chain Reaction is shown in Table 2. The obtained sequences were deposited in GenBank and are listed in Table 3.

Phylogenetic analysis

BioEdit v.7.0 was used to verify the quality of sequences (Hall TA 1999) and MAFFT v.7.215 (http://mafft.cbrc.jp/alignment/server/index.html) was employed to generate single gene alignments (Katoh and Standley 2013). The file format was converted using ALTER (Alignment Transformation Environment)

Table 1. PCR conditions used for ITS, SSU, LSU, *tef*1 and *rpb*2 loci.

Genes	Initial period	Cycles, denaturation, annealing and elongation	Final extension
ITS, LSU, SSU, *tef*1	95°C for 5 min	35 cycles of denaturation at 94 °C for 1 min, annealing at 52°C for 1 min, elongation at 72°C for 1.5 min	72°C for 10 minutes
*rpb*2	95°C for 5 min	35 cycles of denaturation at 95°C for 1 minute, annealing at 54°C for 2 minutes, elongation at 72°C for 1.5 minutes	72°C for 10 minutes

Table 2. Composition of PCR reaction system.

Components	Volumetry	Concentration
2× Tap PCR Mix	12.5 μl	1×
Primer 1	1 μl	10μM μl^{-1}
Primer	1 μl	10μM μl^{-1}
DNA template	1 μl	0.1-0.2 $\mu g\ \mu l^{-1}$
ddH_2O	Up to 25 μl	

Table 3. Taxa and corresponding GenBank accession numbers of sequences used in the phylogenetic analysis of Didymosphaeriaceae, Roussoellaceae and Nigrogranaceae.

Species	Strain	GenBank Accession Numbers					References
		ITS	SSU	LSU	*tef*1	*rpb*2	
Alloconiothyrium camelliae	NTUCC 17-032-1^T	MT112294	MT071221	MT071270	MT232967	–	(Kolařík et al. 2017)
Arthopyrenia sp.	UTHSC DI16–362	LT796905	LN907505	–	LT797145	LT797065	(Crous et al. 2015)
Austropleospora ochracea	KUMCC 20-0020^T	MT799859	MT808321	MT799860	MT872714	–	(Dissanayake et al. 2021)
A. keteleeriae	MFLUCC 18-1551^T	NR_163349	MK347910	NG_070075	MK360045	–	(Mapook et al. 2020)
Biatriospora antibiotica	CCF 1998	LT221894	–	–	–	–	(Kolařík et al. 2017)
B. carollii	CCF 4484^T	LN626657	–	–	LN626668	–	(Kolařík et al. 2017)
B. mackinnonii	E9303e	–	–	–	LN626673	–	(Kolařík et al. 2017)
B. peruviensis	CCF 4485^T	LN626658	–	–	LN626671	–	(Kolařík et al. 2017)
Bimuria omanensis	SQUCC 15280^T	NR_173301	–	NG_071257	MT279046	–	(Wijesinghe et al. 2020)
B. novae-zelandiae	CBS 107.79^T	MH861181	AY016338	AY016356	DQ471087	–	(Vu et al. 2019)
Chromolaenicola nanensis	MFLUCC 17-1477	MN325014	MN325008	MN325002	MN335647	–	(Liu et al. 2014)
C. siamensis	MFLUCC 17-2527^T	NR_163337	MK347866	NG_066311	MK360048	–	(Mapook et al. 2020)
C. thailandensis	MFLUCC 17-1475	MN325019	MN325013	MN325007	MN335652	–	(Liu et al. 2014)
C. lampangensis	MFLUCC 17-1462^T	MN325016	MN325010	MN325004	MN335649	–	(Liu et al. 2014)
Cylindroaseptospora leucaenae	MFLUCC 17-2424	NR_163333	MK347856	NG_066310	MK360047	–	(Mapook et al. 2020)
Deniquelata hypolithi	CBS 146988^T	MZ064429	–	NG_076735	MZ078250	–	(Ariyawansa et al. 2020b)
D. barringtoniae	MFLUCC 16-0271	MH275059	–	MH260291	MH412766	–	(Tibpromma et al. 2018)
Didymocrea sadasivanii	CBS 438.65	MH858658	DQ384066	DQ384103	–	–	(Vu et al. 2019)
Didymosphaeria rubi-ulmifolii	MFLUCC 14-0023^T	–	NG_063557	KJ436586	–	–	(Jayasiri et al. 2019)
Kalmusia erioi	MFLU 18-0832^T	MN473058	MN473046	MN473052	MN481599	–	(Vu et al. 2019)
K. italica	MFLUCC 13-0066^T	KP325440	KP325442	KP325441	–	–	(Vu et al. 2019)
K. variisporum	CBS 121517^T	NR_145165	–	JX496143	–	–	(Wijesinghe et al. 2020)
K. ebuli	CBS 123120^T	KF796674	JN851818	JN644073	–	–	(Dissanayake et al. 2021)
Kalmusibambusa triseptata	MFLUCC 13-0232	KY682697	KY682696	KY682695	–	–	(Tibpromma et al. 2018)
Karstenula rhodostoma	CBS 690.94	–	GU296154	GU301821	GU349067	–	(Crous et al. 2021)
Laburnicola hawksworthii	MFLUCC 13-0602^T	KU743194	KU743196	KU743195	–	–	(Ariyawansa et al. 2014)
Letendraea helminthicola	CBS 884.85	MK404145	AY016345	AY016362	MK404174	–	(Tibpromma et al. 2018)
L. muriformis	MFLUCC 16-0290^T	KU743197	KU743199	KU743198	KU743213	–	(Ariyawansa et al. 2014)
L. padouk	CBS 485.70	–	GU296162	AY849951	–	–	(Zhang et al. 2013)
L. cordylinicola	MFLUCC 11 0148^T	NR_154118	KM214001	NG_059530	–	–	(Wijayawardene et al. 2020)
Montagnula chromolaenicola	MFLUCC 17-1469^T	NR_168866	NG_070157	NG_070948	MT235773	–	(Liu et al. 2014)
M. cirsii	MFLUCC 13 0680	KX274242	KX274255	KX274249	KX284707	–	(Hyde et al. 2020)
M. krabiensis	MFLUCC 16-0250^T	MH275070	MH260343	MH260303	MH412776	–	(Tibpromma et al. 2018)

Species	Strain	GenBank Accession Numbers					References
		ITS	SSU	LSU	*tef*1	*rpb*2	
M. thailandica	MFLUCC 17-1508^T	MT214352	NG_070158	NG_070949	MT235774	–	(Liu et al. 2014)
M. bellevaliae	MFLUCC 14-0924^T	NR_155377	KT443904	KT443902	KX949743	–	(Ariyawansa et al. 2014)
Neoroussoella alishanense	FU31016	MK503816	MK503822	–	MK336181	MN037756	(Verkley et al. 2014)
N. bambusae	MFLUCC 11–0124	KJ474827	KJ474839	–	KJ474848	KJ474856	(Dissanayake et al. 2021)
N. brevispora	KT2313^T	LC014574	AB524460	AB524601	AB539113	–	(Tanaka et al. 2015)
N. brevispora	KT1466	LC014573	AB524459	AB524600	AB539112	–	(Tanaka et al. 2015)
N. heveae	MFLUCC 17–1983	MH590693	MH590689	–	–	–	(Wanasinghe et al. 2018)
N. jonahhulmei	KUMCC 21-0819	ON007044	ON007040	ON007049	ON009134	–	(Wanasinghe et al. 2016)
N. karka	**GMB0494^T**	**OR120445**	**OR120442**	**OR120432**	**OR150020**	–	**This study**
N. karka	**GMB0500**	**OR120438**	**OR120433**	**OR120443**	**OR150021**	–	**This study**
N. kunmingensis	KUMCC 18-0120^T	MK079886	MK079887	MK079889	MK070172	–	(Vu et al. 2019)
N. lenispora	GZCC 16-0020^T	–	KX791431	–	–	–	(Hyde et al. 2020)
N. scabrispora	KT1023	LC014575	AB524452	AB524593	AB539106	–	(Tanaka et al. 2015)
N. solani	CPC 26331^T	KX228261	KX228312	–	–	–	(Wijayawardene et al. 2014)
N. thailandica	MFLUCC 16-0405^T	NR_154255	KY706137	NG_059792	KY706145	–	(Thambugala et al. 2015)
Neokalmusia arundinis	MFLUCC 15-0463^T	NR_165852	NG_068372	NG_068237	KY244024	–	(Thambugala et al. 2015)
Nigrograna antibiotica	CCF 4378^T	JX570932	–	–	JX570934	–	(Kolařík et al. 2018)
Nigrograna cangshanensis	MFLUCC15-0253^T	KY511063	–	–	KY511066	–	(Crous et al. 2015)
N. chromolaenae	MFLUCC 17-1437^T	MT214379	–	–	MT235801	–	(Liu et al. 2014)
N. didymospora	MFLUCC 11-0613	–	KP091435	KP091434	–	–	(Haridas et al. 2020)
N. fuscidula	CBS 141556^T	KX650550	–	–	KX650525	–	(Feng et al. 2019)
N. fuscidula	CBS 141476	KX650547	–	–	KX650522	–	(Feng et al. 2019)
N. hydei	GZCC 19-0050^T	NR_172415	–	–	MN389249	–	(Zhang et al. 2020)
N. impatientis	GZCC 19-0042^T	NR_172416	–	–	MN389250	–	(Zhang et al. 2020)
N. leucaenae	MFLUCC 18–1544	MK347767	MK347984	–	MK360067	MK434876	(Mapook et al. 2020)
N. locuta-pollinis	CGMCC 3.18784	MF939601	–	–	MF939613	–	(Ahmed et al. 2014)
N. locuta-pollinis	LC11690	MF939603	–	–	MF939614	–	(Ahmed et al. 2014)
N. mackinnonii	CBS 674.75^T	NR_132037	–	–	KF407986	–	(Ariyawansa et al. 2015)
N. mackinnonii	E5202H	JX264157	–	–	JX264154	–	(Phukhamsakda et al. 2018)
N. magnoliae	GZCC 17-0057	MF399066	–	–	MF498583	–	(Zhang et al. 2020)
N. magnoliae	MFLUCC 20-0020^T	MT159628	–	–	MT159605	–	(Liu et al. 2014)
N. mycophila	CBS 141478^T	KX650553	–	–	KX650526	–	(Feng et al. 2019)
N. mycophila	CBS 141483	KX650555	–	–	KX650528	–	(Feng et al. 2019)
N. norvegica	CBS 141485^T	KX650556	–	–	–	–	(Feng et al. 2019)
N. obliqua	CBS 141477^T	KX650560	–	–	KX650531	–	(Feng et al. 2019)
N. obliqua	CBS 141475	KX650558	–	–	KX650530	–	(Feng et al. 2019)
N. rhizophorae	MFLUCC 18-0397^T	MN047085	–	–	MN077064	–	(Poli et al. 2020)
N. samueliana	NFCCI-4383^T	MK358817	–	–	MK330937	–	(Poli et al. 2020)
N. schinifolium	**GMB0498^T**	**OR120434**	–	–	**OR150022**	–	**This study**
N. schinifolium	**GMB0504**	**OR120441**	–	–	**OR150023**	–	**This study**
N. thymi	MFLUCC 14-1096^T	KY775576	–	–	KY775578	–	(Crous et al. 2015)
N. trachycarpus	**GMB0499^T**	**OR120437**	–	–	**OR150024**	–	**This study**
N. trachycarpus	**GMB0505**	**OR120440**	–	–	**OR150025**	–	**This study**
N. yasuniana	YU.101026^T	HQ108005	–	–	LN626670	–	(Kolařík et al. 2018)
Occultibambusa pustula	MFLUCC 11-0502^T	KU940126	–	–	–	–	(Crous et al. 2014)
O. bambusae	MFLUCC 13-0855^T	KU940123	–	–	KU940193	–	(Crous et al. 2014)
Paracamarosporium fagi	CPC 24890^T	NR_154318	–	NG_070630	–	–	(Ariyawansa et al. 2014)
P. cyclothyrioides	CBS 972.95	JX496119	AY642524	JX496232	–	–	(Schoch et al. 2009)
P. estuarinum	CBS 109850^T	JX496016	AY642522	JX496129	–	–	(Verkley et al. 2014)
P. hawaiiense	CBS 120025^T	JX496027	EU295655	JX496140	–	–	(Verkley et al. 2014)
P. robiniae	MFLUCC 14–1119^T	KY511142	KY511141	–	KY549682	–	(Crous et al. 2015)
P. rosarum	MFLUCC 17–6054^T	NR_157529	NG_059872	–	MG829224	–	(Hyde et al. 2016)
P. rosicola	MFLUCC 15-0042	NR_157528	MG829153	MG829047	–	–	(Hyde et al. 2016)

Species	Strain	GenBank Accession Numbers					References
		ITS	SSU	LSU	*tef*1	*rpb*2	
Paramassariosphaeria anthostomoides	CBS 615.86	MH862005	GU205246	GU205223	–	–	(Vu et al. 2019)
Paraphaeosphaeria rosae	MFLUCC 17-2547^{T}	MG828935	MG829150	MG829044	MG829222	–	(Hyde et al. 2016)
Pararoussoella mukdahanensis	KUMCC 18-0121	MH453489	MH453485	–	MH453478	MH453482	(Flakus et al. 2019)
Parathyridaria ramulicola	CBS 141479^{T}	KX650565	KX650565	–	KX650536	KX650584	(Feng et al. 2019)
Phaeodothis winteri	CBS 182.58	–	GU296183	GU301857	–	–	(Zhang et al. 2013)
Pseudocamarosporium propinquum	MFLUCC 13-0544^{T}	KJ747049	KJ819949	KJ813280	–	–	(Thambugala et al. 2017)
Pseudodidymocyrtis lobariellae	KRAM Flakus 25130^{T}	NR_169714	NG_070349	NG_068933	–	–	(Tanaka et al. 2015)
Pseudoneoconiothyrium euonymi	CBS 143426^{T}	MH107915	MH107961	–	–	MH108007	(Valenzuela-Lopez et al. 2017)
Pseudopithomyces entadae	MFLUCC 17-0917^{T}	–	MK347835	NG_066305	MK360083	–	(Mapook et al. 2020)
Pseudoroussoella chromo-laenae	MFLUCC 17–1492^{T}	MT214345	MT214439	–	MT235769	–	(Liu et al. 2014)
P. elaeicola	MFLUCC 15–0276a	MH742329	MH742326	–	–	–	(Liu et al. 2014)
P. kunmingnensis	MFLUCC 17-0314	MF173607	MF173606	MF173605	–	–	(Mapook et al. 2020)
P. pteleae	MFLUCC 17-0724^{T}	NR_157536	MG829166	MG829061	MG829233	–	(Hyde et al. 2016)
P. rosae	MFLUCC 15-0035^{T}	MG828953	MG829168	MG829064	–	–	(Hyde et al. 2016)
P. ulmi-minoris	MFLUCC 17-0671^{T}	NR_157537	MG829167	MG829062	–	–	(Hyde et al. 2016)
Roussoella acaciae	CBS:138873^{T}	KP004469	KP004497	–	–	–	(Karunarathna et al. 2019)
R. aquatic	MFLUCC 18-1040^{T}	NR171975	NG073797	–	–	–	(Liu et al. 2014)
R. chiangraina	MFLUCC 10-0556^{T}	NR155712	NG059510	–	–	–	(Dissanayake et al. 2021)
R. doimaesalongensis	MFLUCC 14-0584^{T}	NR165856	NG068241	–	KY651249	KY678394	(Thambugala et al. 2015)
R. doimaesalongensis	**GMB0497**	**OR116188**	**OR117732**	–	**OR150026**	–	**This study**
R. doimaesalongensis	**GMB0503**	**OR120435**	**OR120444**	–	**OR150027**	–	**This study**
R. elaeicola	MFLUCC 15-15-0276a	MH742329	MH742326	–	–	–	(Crous et al. 2015)
R. euonymi	CBS:143426^{T}	MH107915	MH107961	–	–	MH108007	(Valenzuela-Lopez et al. 2017)
R. guttulata	MFLUCC 20-0102^{T}	NR172428	NG075383	–	–		(Senwanna et al. 2018)
R. hysterioides	CBS 546.94	MH862484	MH874129	–	KF443399	KF443392	(Vilgalys et al. 1990)
R. intermedia	CBS 170.96	KF443407	KF443382	–	KF443398	KF443394	(Crous et al. 2013)
R. japanensis	MAFF 239636^{T}	NR155713	–	–	–	–	(Dissanayake et al. 2021)
R. kunmingensis	HKAS 101773^{T}	MH453491	MH453487	–	MH453480	MH453484	(Flakus et al. 2019)
R. magnatum	MFLUCC 15-0185^{T}	–	KT281980	–	–	–	(Jiang et al. 2019)
R. mangrovei	MFLU 17-1542^{T}	MH025951	MH023318	–	MH028246	MH028250	(Jaklitsch and Voglmayr 2016)
R. margidorensis	MUT 5329^{T}	NR169906	MN556322	–	MN605897	MN605917	(Tibpromma et al. 2017)
R. mediterranea	MUT5369^{T}	KU314947	MN556324	–	MN605899	MN605919	(Tibpromma et al. 2017)
R. mexicana	CPC 25355^{T}	KT950848	KT950862	–	–	–	(Crous et al. 2015a)
R. mukdahanensis	MFLU 11-0237^{T}	NR155722	–	–	–	–	(Crous et al. 2014)
R. multiplex	GMB0316^{T}	ON479891	–	ON479892	–	–	(Dong et al. 2020)
R. neopustulans	MFLUCC 11-0609^{T}	KJ474833	KJ474841	–	KJ474850	–	(Dissanayake et al. 2021)
R. neopustulans	**GMB0496**	**OR120436**	**OR120446**				**This study**
R. neopustulans	**GMB0502**	**OR116176**	**OR117714**				**This study**
R. nitidula	MFLUCC 11-0634	KJ474834	KJ474842	–	KJ474851	KJ474858	(Dissanayake et al. 2021)
R. padinae	MUT 5503^{T}	–	MN556327	–	MN605902	MN605922	(Tibpromma et al. 2017)
R. percutanea	CBS 868.95	KF322118	KF366449	–	KF407987	KF366452	(Ahmed et al. 2014a)
R. pseudohysterioides	GMBC0009^{T}	MW881445	MW881451	–	–	MW883345	(Zhang et al. 2020)
R. pseudohysterioides	**GMB0495**	**OR116175**	**OR117737**	–	**OR150028**	–	**This study**
R. pseudohysterioides	**GMB0501**	**OR120447**	**OR120439**	–	**OR150029**	–	**This study**
R. pustulans	KT 1709	–	AB524623	–	AB539116	AB539103	(Zhang et al. 2020)
R. scabrispora	MFLUCC 14-0582	KY026583	KY000660	–	–	–	(Zhang et al. 2020)
R. siamensis	MFLUCC 11-0149^{T}	KJ474837	KJ474845	–	KJ474854	KJ474861	(Dissanayake et al. 2021)
R. thailandica	MFLUCC 11-0621^{T}	KJ474838	KJ474846	–	–	–	(Dissanayake et al. 2021)
R. tuberculata	MFLUCC 13-0854^{T}	KU940132	KU863121	–	KU940199		(Crous et al. 2014)
R. verrucispora	CBS 125434^{T}	KJ474832	–	–	–	–	(Dissanayake et al. 2021)

Species	Strain	GenBank Accession Numbers					References
		ITS	SSU	LSU	*tef*1	*rpb*2	
R. yunnanensis	HKAS 101762	MH453492	MH453488	–	MH453481	–	(Flakus et al. 2019)
Roussoellopsis macrospora	MFLUCC 12-0005	–	KJ474847	–	KJ474855	KJ474862	(Dissanayake et al. 2021)
R. tosaensis	KT 1659	–	AB524625	–	AB539117	AB539104	(Zhang et al. 2020)
Setoarthopyrenia chromolaenae	MFLUCC 17–1444	MT214344	MT214438	–	MT235768	MT235805	(Liu et al. 2014)
Spegazzinia deightonii	yone 212	–	AB797292	AB807582	AB808558	–	(Tanaka et al. 2015)
S. radermacherae	MFLUCC 17-2285^{T}	MK347740	MK347848	MK347957	MK360088	–	(Mapook et al. 2020)
S. tessarthra	NRRL 54913	JQ673429	AB797294	AB807584	AB808560	–	(Tanaka et al. 2015)
Thyridaria acaciae	CBS 138873	KP004469	KP004497	–	–	–	(Liu et al. 2014)
T. broussonetiae	CBS 141481	NR_147658	KX650568	–	KX650539	KX650586	(Karunarathna et al. 2019)
Torula herbarum	CBS 111855	KF443409	KF443386	–	KF443403	KF443396	(Crous et al. 2013)
T. hollandica	CBS 220.69	KF443406	KF443384	–	–	KF443393	(Crous et al. 2013)
Tremateia arundicola	MFLU 16-1275	KX274241	KX274254	KX274248	KX284706	–	(Hyde et al. 2020)
T. chromolaenae	MFLUCC 17-1425^{T}	NR_168868	NG_070160	NG_068710	MT235778	–	(Tanaka et al. 2015)
T. guiyangensis	GZAAS01	KX274240	KX274253	KX274247	KX284705	–	(Hyde et al. 2020)
T. murispora	GZCC 18-2787	NR_165916	MK972750	MK972751	MK986482	–	(Feng et al. 2019)
T. thailandensis	MFLUCC 17-1430^{T}	NR_168869	NG_070161	NG_068711	MT235781	–	(Liu et al. 2014)
Verrucoconiothyrium nitidae	CBS:119209	EU552112	–	EU552112	–	–	(Wanasinghe et al. 2018)
Xenocamarosporium acaciae	CPC 24755^{T}	NR_137982	–	NG_058163	–	–	(Crous et al. 2015b)
Xenoroussoella triseptata	MFLUCC 17–1438	MT214343	MT214437	–	MT235767	MT235804	(Liu et al. 2014)

Notes: Type specimens or Ex-type specimens are marked with T; "–": indicates no sequence available in GenBank; newly-generated sequences are indicated in bold.

Abbreviations: CBS: Centraalbureau voor Schimmelcultures, Utrecht, The Netherlands; **CPC:** Culture collection of Pedro Crous, housed at the Westerdijk Fungal Biodiversity Institute; **GMB:** Culture collection of Guizhou Medical University; **HKAS:** Herbarium of Cryptogams Kunming Institute of Botany Academia Sinica, Chinese Academy of Sciences, Kunming, China; **HKUCC:** Hong Kong University Culture Collection; **KT:** K. Tanaka; **KUMCC:** Kunming Institute of Botany Culture Collection, Chinese Science Academy, Kunming, China; **MAFF:** Ministry of Agriculture, Forestry and Fisheries, Japan; **MFLUCC:** Mae Fah Luang University Culture Collection, Chiang Rai, Thailand; **NFCCI:** National Fungal Culture Collection of India; **Others:** information not available.

(http://www.sing-group.org/ALTER/). Maximum Likelihood (ML) analyses and Bayesian posterior probabilities (BYPP), based on a combination of ITS, LSU, *tef*1 and *rpb*2 sequence data, were performed using RAxML-HPC BlackBox and MrBayes v. 3.2.7a tools in the CIPRES Science Gateway platform (Liang et al. 2020). GTR+I+G was estimated as the best-fit substitution model by ModelTest2 on XSEDE v.2.1.6. (Posada and Crandall 1998).

Bayesian Inference (BI) analysis was conducted using MrBayes v.3.2.7a (Ronquist et al. 2012) and posterior probabilities (PP) were determined through Markov Chain Monte Carlo sampling (MCMC). Six simultaneous Markov chains for 3,000,000 generations were run and trees were sampled every 1,000th generation.

The trees were visualised using FigTree v,1.4.4, and formatted using Adobe Illustrator CS v.6. Branches with Maximum-Likelihood bootstrap values (MLBP) equal to or greater than 75% and Bayesian posterior probabilities (BYPP) greater than 0.95 are indicated. The combined loci alignment and resulting phylogenetic trees were submitted to TreeBASE (https://www.treebase.org, submission number: ID 30482; ID 30483; ID 30484).

Results

Phylogenetic analyses

Phylogenetic analyses of Didymosphaeriaceae (Fig. 1), Roussoellaceae (Fig. 2), and Nigrogranaceae (Fig. 3) were performed separately, with corresponding parameters presented in Table 4.

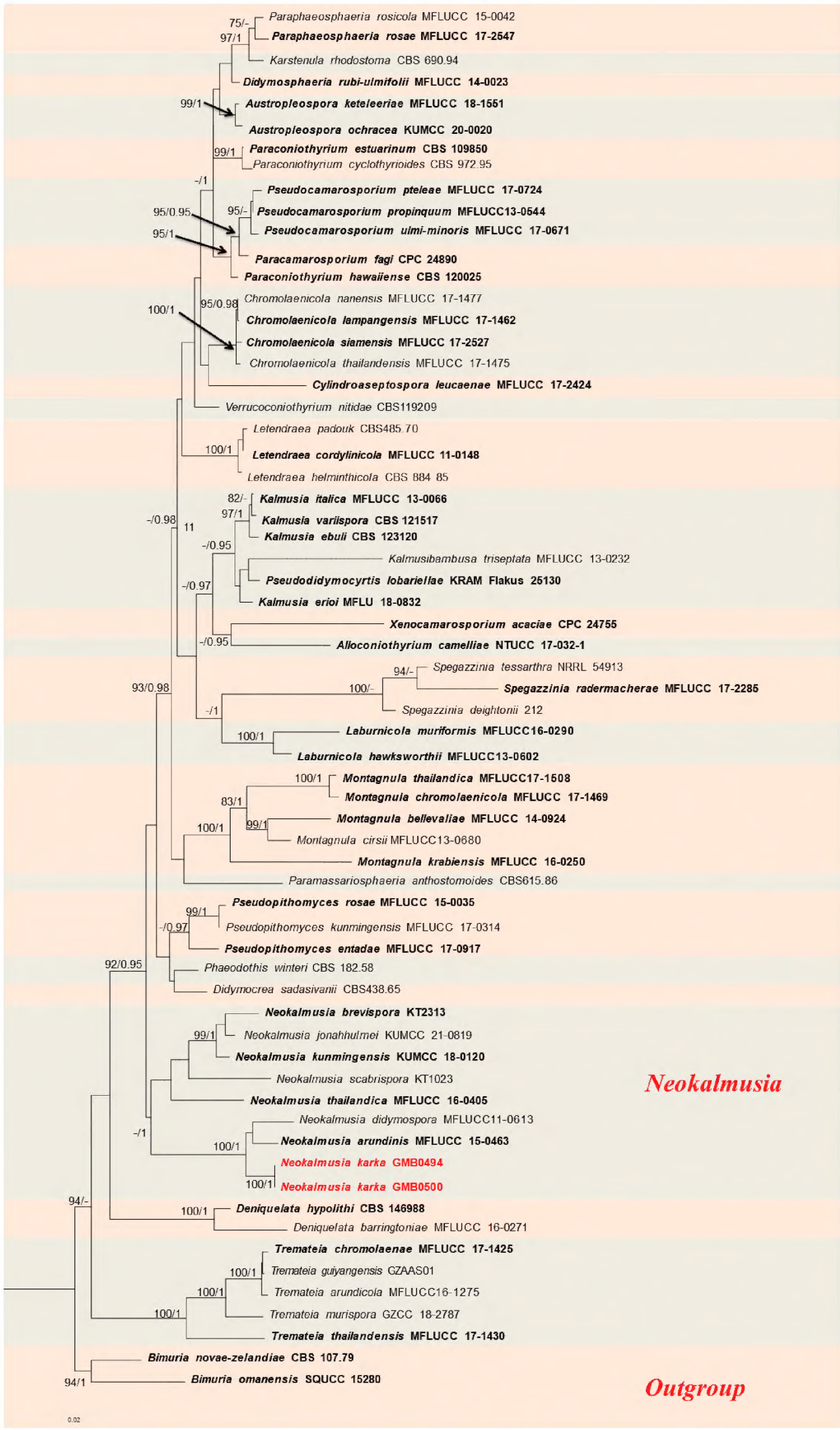

Figure 1. RAxML phylogram of Didymosphaeriaceae, based on a combined dataset of partial ITS, LSU, SSU and *tef*1 DNA sequences. The tree is rooted by *Bimuria novae-zelandiae* (CBS 107.79) and *Bimuria omanensis* (SQUCC 15280). Bootstrap supports ML (MLB ≥ 75%) and Bayesian posterior probabilities (BYPP ≥ 0.95) are given as MLB/BYPP above the branches. Sequences from newly-generated isolates are in red, bold letters, while those of ex-type isolates are shown in black, bold letters.

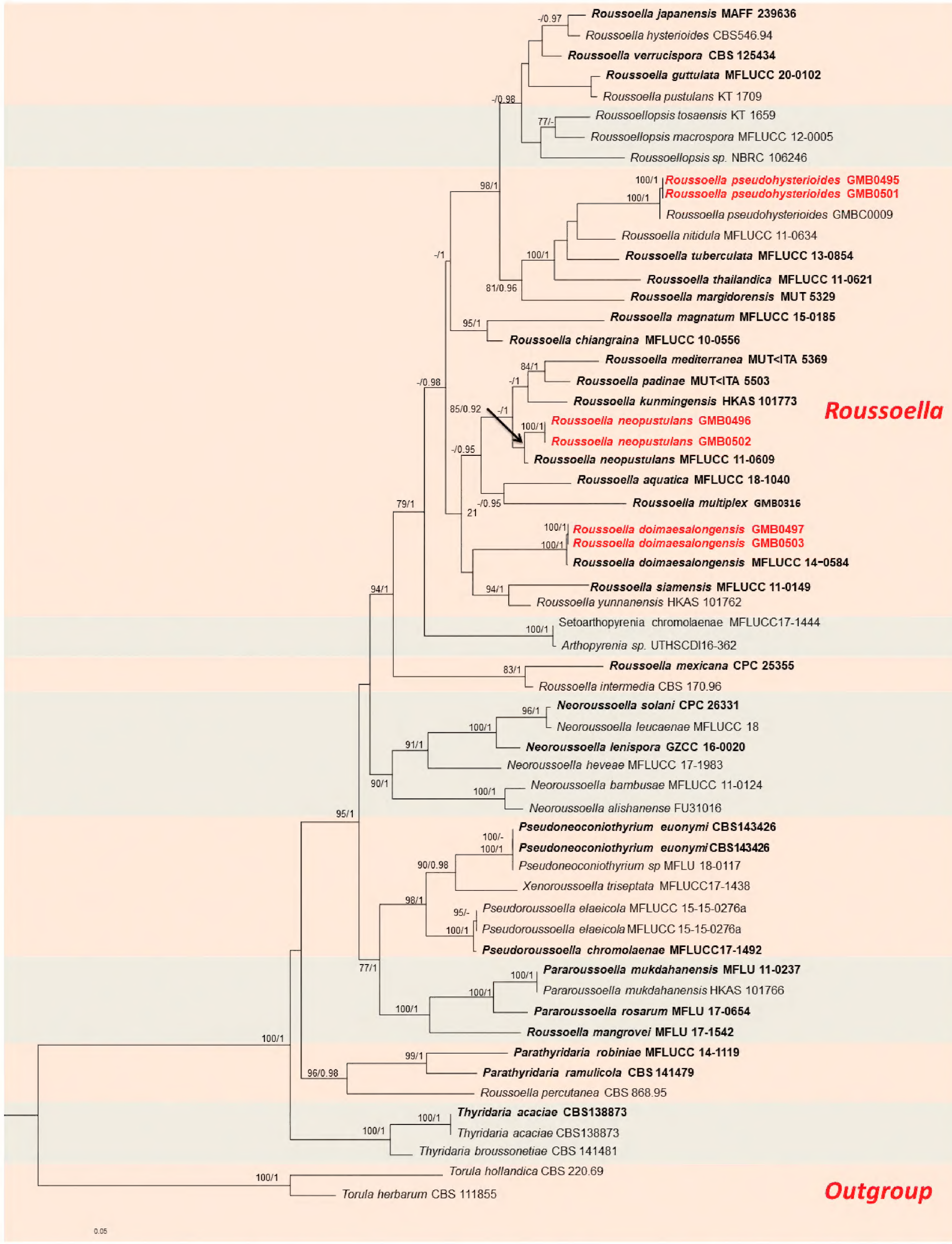

Figure 2. RAxML phylogram of Roussoellaceae, based on a combined dataset of partial ITS, LSU, *tef*1 and *rpb*2 DNA sequences. The tree is rooted by *Torula hollandica* (CBS 220.69) and *T. herbarum* (CBS 111855). Bootstrap supports ML (MLB ≥ 75%) and Bayesian posterior probabilities (BYPP ≥ 0.95) are given as MLB/BYPP above the branches. Sequences from newly-generated isolates are in red, bold letters, while those of ex-type isolates are shown in black, bold letters.

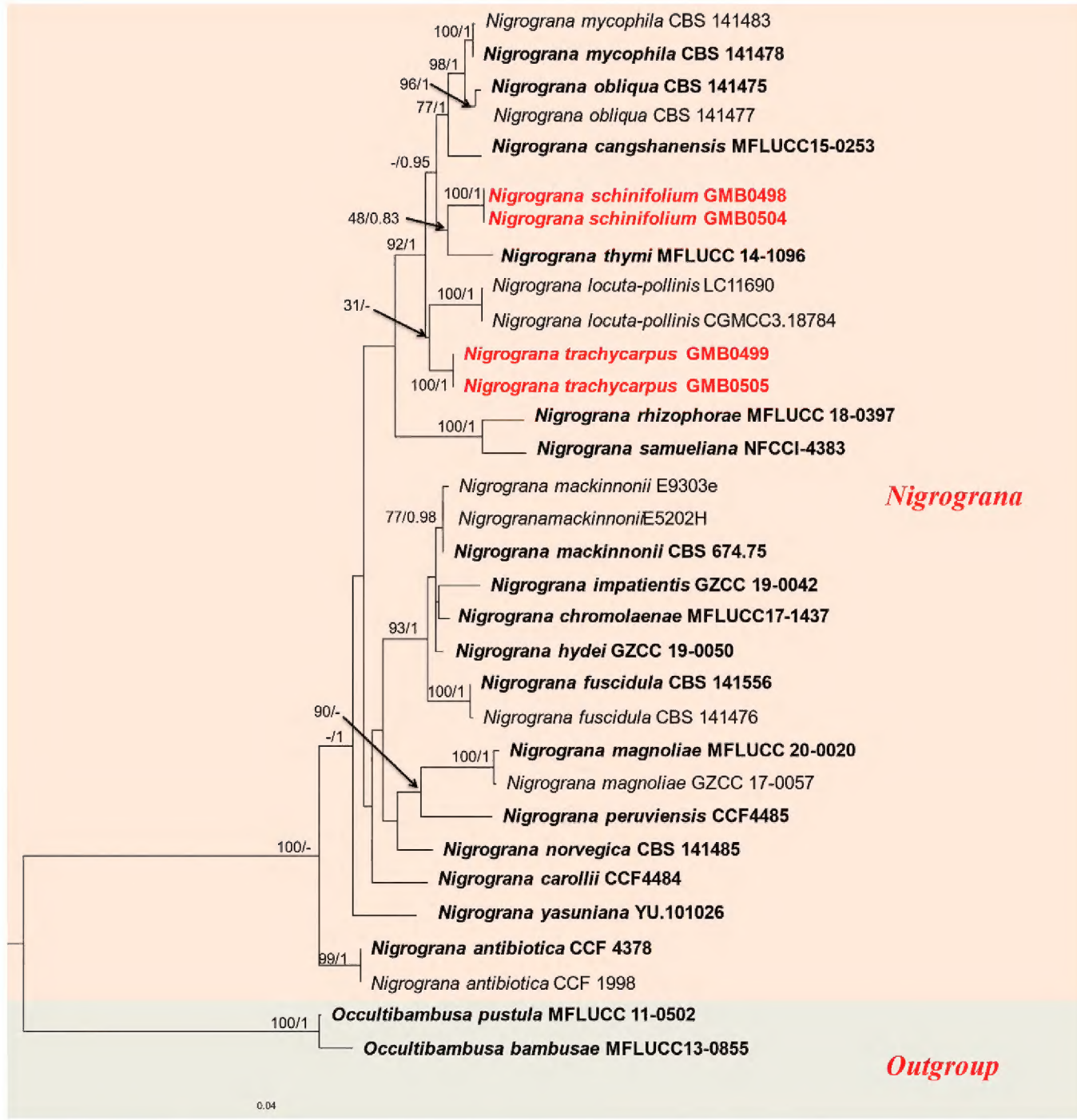

Figure 3. RAxML phylogram of Nigrogranaceae, based on a combined dataset of ITS and *tef*1 DNA sequences. The tree is rooted by *Occultibambusa pustula* (MFLUCC 11-0502) and *O. bambusae* (MFLUCC 13-0855). Bootstrap supports ML (MLB ≥ 75%) and Bayesian posterior probabilities (BYPP ≥ 0.95) are given as MLB/BYPP above the branches. Sequences from newly-generated isolates are in red, bold letters, while those of ex-type isolates are shown in black, bold letters.

Table 4. Results of Maximum-Likelihood (ML) and Bayesian (BI) analyses for each sequenced dataset.

Analyses		Didymosphaeriaceae	Roussoellaceae	Nigrogranaceae
Number of taxa		64	59	32
Gene regions		ITS, LSU, SSU and *tef*1	ITS, LSU, *tef*1 and *rpb*2	ITS and *tef*1
Number of character positions (including gaps)		2423	2267	868
ML optimisation likelihood value		-13324.603084	-16237.062124	-3695.409391
Distinct alignment patterns in the matrix		584	773	240
Number of undetermined characters or gaps (%)		14.26%	27.45%	7.87%
Estimated base frequencies	A	0.237970	0.240773	0.229686
	C	0.246811	0.255815	0.293625
	G	0.277468	0.276383	0.242370
	T	0.237752	0.227030	0.234319
Substitution rates	AC	1.764988	2.186105	1.598706
	AG	2.187844	5.410475	2.533043
	AT	1.416956	2.441301	1.640025
	CG	1.132266	1.384067	0.752494
	CT	7.848138	11.885781	8.062830
	GT	1.000000	1.000000	1.000000
Proportion of invariable sites (I)		0.595845	0.544120	0.487317
Gamma distribution shape parameter (a)		0.516792	0.502253	0.634309
Number of generated trees in BI		14806	10678	9932
Average standard deviation of split frequencies		0.006852	0.004431	0.004939

Taxonomy

Didymosphaeriaceae Munk, 1953

***Neokalmusia* Ariyawansa & K.D. Hyde, Fungal Diversity 68: 92 (2014b)**
MycoBank No: 550700

Notes. *Neokalmusia* was established by Ariyawansa et al. (2014b) to accommodate two bambusicolous taxa, *N. brevispora* and *N. scabrispora*, previously referred to *Kalmusia*. Members of *Neokalmusia* are characterised by solitary sphaeroid ascomata, a peridium of small pseudoparenchymatous cells, clavate basal asci with very long pedicels, very thin pseudoparaphyses and distoseptate, smooth-walled ascospores (Ariyawansa et al. 2014b; Zhang et al. 2020a). In this study, we introduce a new species of *Neokalmusia*, based on a combination of morphological and molecular analyses (Fig. 1).

***Neokalmusia karka* H. M. Hu & Q. R. Li, sp. nov.**
MycoBank No: 851046
Fig. 4

Type material. ***Holotype*:** GMB0494.

Etymology. In reference to the host, *Phragmites karka* (Retz.) Trin. ex Steud.

Description. Saprobic on dead culms of *P. karka*.

Sexual morph: ***Clypeus*** visible as black dots on the host surface, breaking through slightly raised cracks at the centre. ***Ascomata*** 241–386 × 161–231 µm (average = 375 × 197 µm, n = 5), smooth, semi-immersed, scattered, solitary or in small groups, black, oval, with ostiole. ***Peridium*** 12–20 µm wide, composed of a few layers of thin-walled, brown to dark brown, cells of textura angularis. ***Hamathecium*** comprising 1.5–2.8 µm wide, numerous, cellular, pseudoparaphyses, embedded in a mucilaginous matrix. ***Asci*** 80–109 × 10–14 µm (average = 95 × 11.4 µm, n = 15), 8-spored, bitunicate, fissitunicate, cylindrical-clavate, with bulbous pedicel, apically rounded with an indistinct ocular chamber, with a J-subapical ring. ***Ascospores*** 14–17 × 4–6 µm (average = 15.8 × 5.3 µm, n = 30), overlapping 1–2-seriate, fusiform, pale brown to brown, 1-septate, constricted at the septum, often enlarged near septum in the upper cell, distinctly verrucose on the surface, without a mucilaginous sheath. **Asexual morph:** undetermined.

Culture characters. After 4 weeks of cultivation at 25 °C, the colonies on PDA measure around 2–2.5 cm in diameter. The surface appears smooth to velvety with an entire or slightly irregular margin, ranging from white to grey olivaceous. The colour is white near the margin with dense circular to filamentous growth. The reverse side of the colonies black to greenish-olivaceous.

Specimens examined. China, Guizhou Province, Zunyi City, Suiyang County, Kuanqwashui Nature Reserve (28°31'51.04"N, 107°9'33.65"E), 1544 m elev., on decaying culms, 12 October 2022, Y.P Wu and H.M Hu, 2022KKS49 (GMB0494, holotype; GMBC0494, ex-type; KUN-HKAS 129179, isotype).

Other examined material. China, Guizhou Province, Huaxi District, Shilihetan Wetland Park (26°41'34.3"N, 106°67'68.8"E), 1500 m elev., on decaying culms, 8 October 2022, Y.P Wu and H.M Hu, 2022SLZH11 (GMB0500; GMBC0500, living culture).

Figure 4. *Neokalmusia karka* (GMB0494, holotype) **A** type specimen **B, C** appearance of ascomata on substrate **D, E** longitudinal section of an ascoma **F** peridium **G** pseudoparaphyses **H–K** asci **L–N** ascospores **O, J** ascus subapical ring in Melzer's Reagent. Scale bars: 0.5 mm (**B–D**); 10 µm (**E–O**).

Notes. This fungus shares morphological characters similar to *Neokalmusia* in having immersed ascomata, a clypeus-like structure composed of thin-walled cells and verrucose ascospores (Tanaka et al. 2009; Ariyawansa et al. 2014b). Other than *Neokalmusia karka*, only two species, *N. arundinis* Thambug. & K.D. Hyde and *N. didymospora* D.Q. Dai & K.D. Hyde have been reported with 1-septate ascospores. However, *N. karka* can be distinguished, based on differences in asci size (*N. karka*, 80–109 × 10–14 µm; *N. arundinis* 60–85 × (7.5–) 8.5–10.5 µm; *N. didymospora* 125–160 × 9.5–14 µm) and the obvious oval shape of its ascomata (Wanasinghe et al. 2018; Flakus et al. 2019). In our phylogram, *Neokalmusia karka* formed a well-supported separate clade (100% ML, 1 BYPP; Fig. 1) in a sister relationship with *N. arundinis* and *N. didymospora*. The macro and micro-morphological differences and phylogenetic analyses support the recognition of *N. karka* as a new species (Fig. 1).

Roussoellaceae Jian K. Liu, Phook., D.Q. Dai & K.D. Hyde 2014

***Roussoella* Sacc., Atti Inst. Veneto Sci. lett., ed Arti, Sér. 6 6: 410 (1888)**
MycoBank No: 541317

Notes. the genus *Roussoella* was introduced by Saccardo et al. (1888), with *R. nitidula* Sacc. & Paol. as the type species, which was collected from bamboo in Malaysia. This family is characterised as having semi-immersed to immersed, solitary or gregarious, clypeate ascostromata containing trabeculate pseudoparaphyses embedded in a gel matrix, long cylindrical to clavate bitunicate asci with or without obvious fissitunicate dehiscence and brown, 2-celled ornamented ascospores (Liu et al. 2014). In this study, we introduce three new records of *Roussoella* species, based on morpho-anatomical and molecular analyses (Fig. 2).

***Roussoella pseudohysterioides* D.Q. Dai & K.D. Hyde, in Dai et al., Fungal Diversity 82(1): 37 (2017)**
MycoBank No: 552026
Fig. 5

Descriptions. See Dai et al. (2017).

Specimen examined. CHINA, Guizhou Province, Huaxi District, Shilihetan Wetland Park (26°43'34.3"N, 106°67'68.8"E), 1542 m elev., on decaying bamboo, 8 October 2022, Y.P Wu and H.M Hu, 2022SLZH6 (GMB0495; GMBC0495, living culture).

Notes. Phylogenetic analyses of the combined ITS, LSU, *tef*1 and *rpb*2 gene sequences showed that the sequence from our 2022SLZH6 collection clusters together with *Roussoella pseudohysterioides* (MFLU 15-1209), with strong support (100% ML, 1 BYPP; Fig. 2). The morphological characteristics of our specimen are also consistent with those of *R. pseudohysterioides*, which was originally described from decaying bamboo culms in Thailand (Dai et al. 2017). In China, it had previously been reported from Yunnan Province (Jiang et al. 2019). This is the second report of this species in China, representing a new record for Guizhou Province.

Figure 5. *Roussoella pseudohysterioides* (GMB0495) **A** specimen **B, C** appearance of ascomata on substrate **D** cross-section of ascostromata **E** longitudinal section of an ascoma **F** peridium **G** pseudoparaphyses **H–I** asci **J–M** ascospores. Scale bars: 0.5 mm (**B–D**); 10 μm (**E–M**).

Roussoella neopustulans **D.Q. Dai, J.K. Liu & K.D. Hyde, in Liu et al. Phytotaxa 181(1): 15 (2014)**

MycoBank No: 550664

Fig. 6

Descriptions. See Liu et al. (2014).

Specimens examined. China, Guizhou Province, Huaxi District, Guiyang Huaxi National Urban Wetland Park (26°2'2.34"N, 106°34'16.22"E), on dead branch

of bamboo, 12 October 2022, 1130 m elev., Y.P Wu and H.M Hu, 2022HX25 (GMB0496; GMBC0496, living culture).

Notes. The sequence of our *Roussoella neopustulans* (2022HX25) forms a well-supported clade (85% ML, 0.92 BYPP; Fig. 2) with *R. neopustulan* (MFLUCC 11-0609). *Roussoella neopustulans* was originally introduced by Liu et al. (2014), with a description of the sexual morph only. Dai et al. (2017) provided a comprehensive description and illustrations for both the sexual and asexual morphs of this species. Our collection exhibits identical morphological characteristics to those detailed by Dai et al. (2017). This is the first report of this species in China.

Roussoella doimaesalongensis **Thambug. & K.D. Hyde, Mycosphere 8 (4): 782 (2017)**

MycoBank No: 553169

Fig. 7

Descriptions. See Thambugala et al. (2017).

Specimen examined. CHINA, Guizhou Province, Huaxi District, Shilihetan Wetland Park (26°23'23.4"N, 106°67'56.4"E), 1511 m elev., on dead bamboo branches, 8 October 2022, Y.P Wu and H.M Hu, 2022SLHT14 (GMB0497; GMBC0497, living culture).

Notes. In our phylogram (Fig. 2), the sequence of our collection clustered with *Roussoella doimaesalongensis* with robust support (100% ML, 1 BYPP). *Roussoella doimaesalongensis* was originally found on decaying bamboo culms in Thailand (Thambugala et al. 2017). Morphologically, our specimens match the description provided by Thambugala et al. (2017) and this species was first reported in China by Seong et al. (2022).

Nigrogranaceae Jaklitsch & Voglmayr, 2016

Nigrograna **Gruyter, Verkley & Crous, Stud. Mycol. 75: 31 (2012) [2013]**

MycoBank No: 564794

Notes. *Nigrograna* was described by De Gruyter et al. (2012) as a monotypic genus. *Nigrograna* is characterised by black ascomata, clavate, short pedicellate asci and pale to chocolate brown, asymmetric, fusoid to narrowly ellipsoid, septate ascospores (Zhang et al. 2020a).

Nigrograna schinifolium **H. M. Hu & Q. R. Li, sp. nov.**

MycoBank No: 849204

Fig. 8

Type material. ***Holotype*****.** GMB0498.

Etymology. With reference to the host, *Zanthoxylum schinifolium* Sieb. & Zucc.

Description. Saprobic on dead stem of *Z. Schinifolium.*

Sexual morph: ***Ascomata*** 198–320 µm wide, 105–160 µm high, solitary or aggregated in small groups, black, semi-immersed, appearing as slightly raised regions. ***Ostioles*** are black, lined with paraphyses. ***Peridium*** 26–39 µm wide,

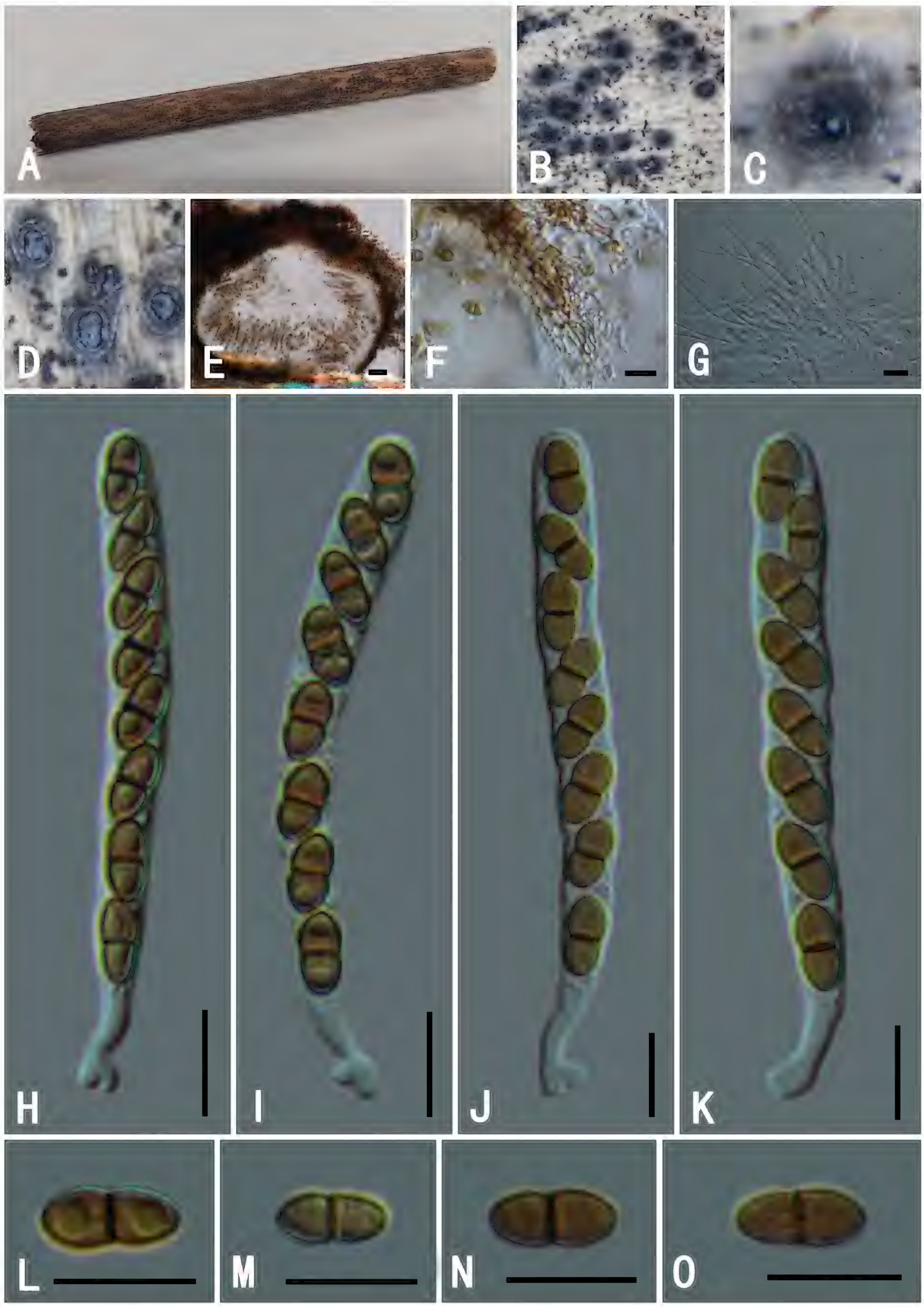

Figure 6. *Roussoella neopustulans* (GMB0496) **A** specimen **B, C** appearance of ascomata on substrate **D** cross-section of ascostromata **E** longitudinal section of an ascoma **F** peridium **G** pseudoparaphyses **H–K** asci **L–O** ascospores. Scale bars: 0.5 mm (**B–D**); 10 µm (**E–O**).

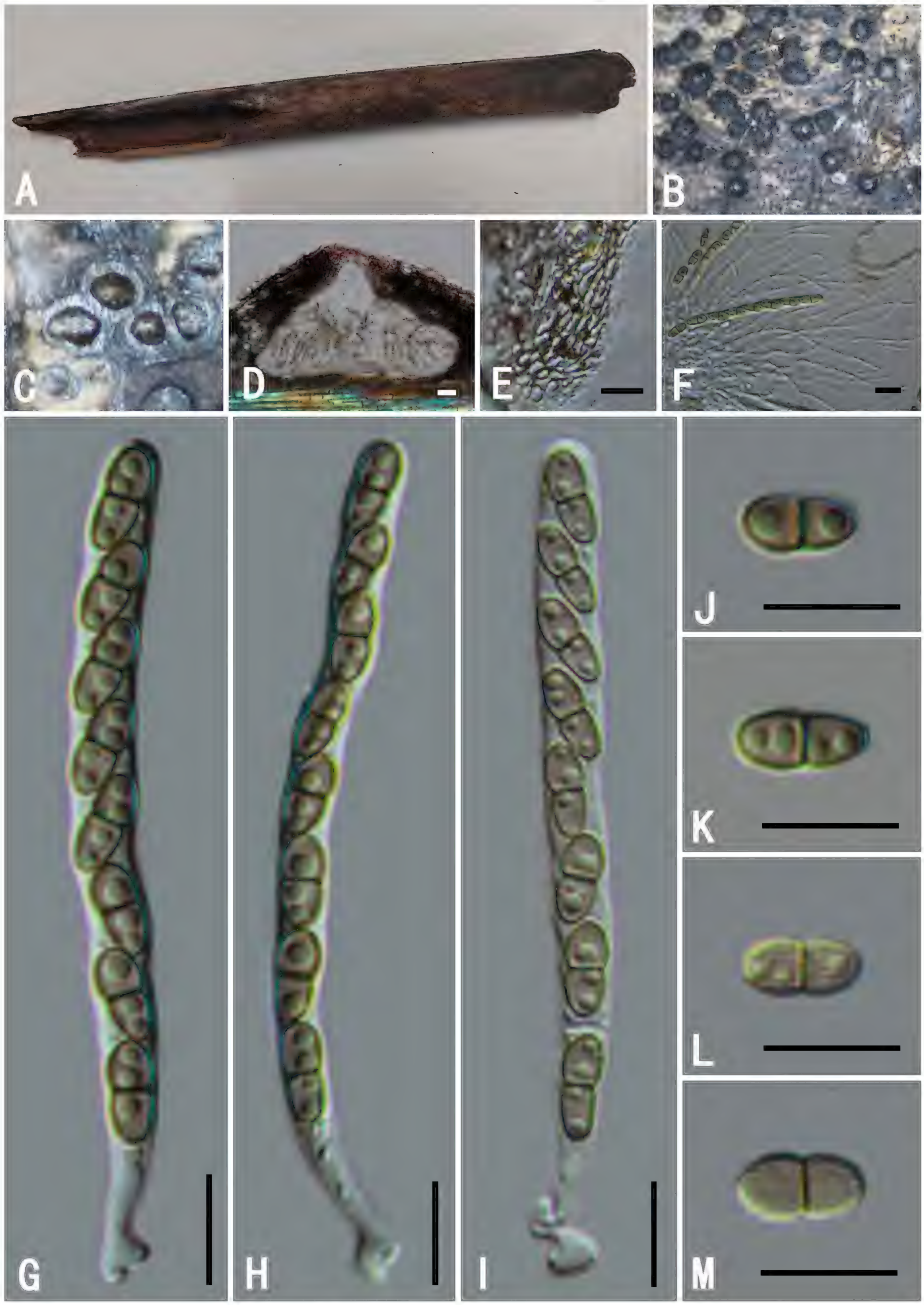

Figure 7. *Roussoella doimaesalongensis* (GMB0497) **A** specimen **B** appearance of ascomata on substrate **C** cross-section of ascostromata **D** longitudinal section of an ascoma **E** peridium **F** pseudoparaphyses **G–I** asci **J–M** ascospores. Scale bars: 0.5 mm (**B–C**); 10 µm (**D–M**).

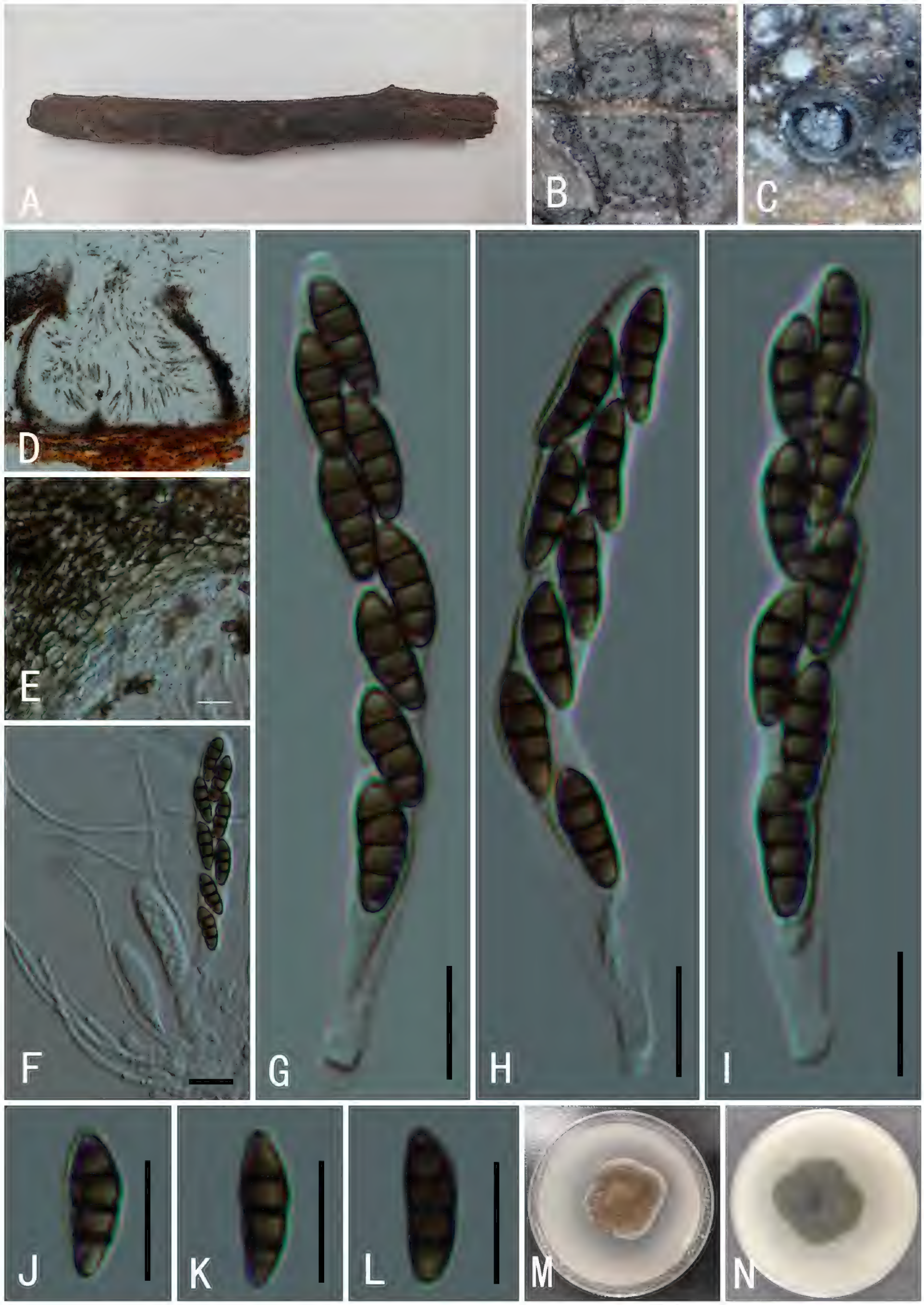

Figure 8. *Nigrograna schinifolium* (GMB0498) **A** specimen **B** appearance of ascomata on substrate **C** cross-section of ascomata **D** longitudinal section of an ascoma **E** peridium **F** pseudoparaphyses **G–I** asci **J–L** ascospores **M, N** culture on PDA. Scale bars: 0.5 mm (**B–C**); 10 μm (**D–L**).

comprising several fused layers of "textura angularis", thin-walled and pale brown at the interior, becoming darker and thicker-walled to the outside. ***Hamathecium*** comprising 1–2 µm wide, cylindrical to filiform, septate, branched, pseudoparaphyses, embedded in a gelatinous matrix. ***Asci*** 44–59 × 8–10 µm (average = 51.5 × 9.3 µm, n = 25), 8-spored, bitunicate, fissitunicate, cylindrical to broadly filiform, with a short stipe and knob-like base, apically rounded with a minute ocular chamber. ***Ascospores*** 10–14 × 2.8–4 µm (average = 11.6 × 3.3 µm, n = 40), broadly fusiform to inequilaterally ellipsoid, with the second cell slightly enlarged, straight or slightly curved, with obtuse to rounded ends, hyaline when immature, becoming brown to dark brown at maturity, 3-eusep-tate, slightly constricted at the median septum. **Asexual morph:** undetermined.

Culture characters. After 4 weeks at 25 °C, colonies on PDA have a diameter of 2–2.5 cm and are circular, slightly raised to umbonate and dull with an entire edge. They appear floccose and smooth and droplets can be observed due to cellular respiration, water formation or antibiotic production. Colonies from the upper region have brown to cream-coloured margins and blackish-brown centres, while their reverse is white to yellowish-brown at the margin and black-ish-brown in the centre.

Specimen examined. China, Guizhou Province, Qiannan Prefecture, San-du Shui Autonomous County, Yao Man Mountain National Forest Park (25°94'18.76"N, 107°95'70.09"E), 563 m elev., on branches of *Zanthoxylum schinifolium*, 28 September 2022, Y.P. Wu, 2022YRS36 (GMB0498, holotype, GMBC0498, ex-type; KUN-HKAS 12983, isotype).

Other examined material. China, Guizhou Province, Huaxi District, Shili-hetan Wetland Park (26°23'13.4"N, 106°66'56.4"E), 1501 m elev., on branches of *Zanthoxylum schinifolium*, 8 October 2022, Y.P Wu and H.M Hu, 2022SLHT44 (GMB0504; GMBC0504, living culture).

Notes. *Nigrograna schinifolium* and *N. thymi* Mapook et al. form a monophy-letic clade with moderate support (MPBP 48%, BYPP 0.83, Fig. 3). However, *N. schinifolium* is distinguished by having 3-septate ascospores (Hyde et al. 2017). Morphologically, *N. schinifolium* can be distinguished from other spe-cies of *Nigrograna* by its shorter asci and ascospores (Hyde et al. 2017; Zhao et al. 2018; Zhang et al. 2020a). Our research confirms *N. schinifolium* is a new species.

Nigrograna trachycarpus **H. M. Hu & Q. R. Li, sp. nov.**
MycoBank No: 849205
Fig. 9

Type material. ***Holotype*****:** GMB0499.

Etymology. Named after the host genus *Trachycarpus* from which the fungus was isolated.

Description. Saprobic or parasitic on dead culms of *Trachycarpus* sp.

Sexual morph: ***Ascomata*** 160–380 µm wide, 100–210 µm high, pyriform to globose, scattered or clustered in small groups, black, immersed, the base remaining immersed in the substrate, smooth, with ostiole. ***Ostiole*** single, central, flattened, with a short neck, without paraphyses. ***Peridium*** 22–34 µm wide, multi-layered, composed of 4–6 rows of heavily pigmented, light brown

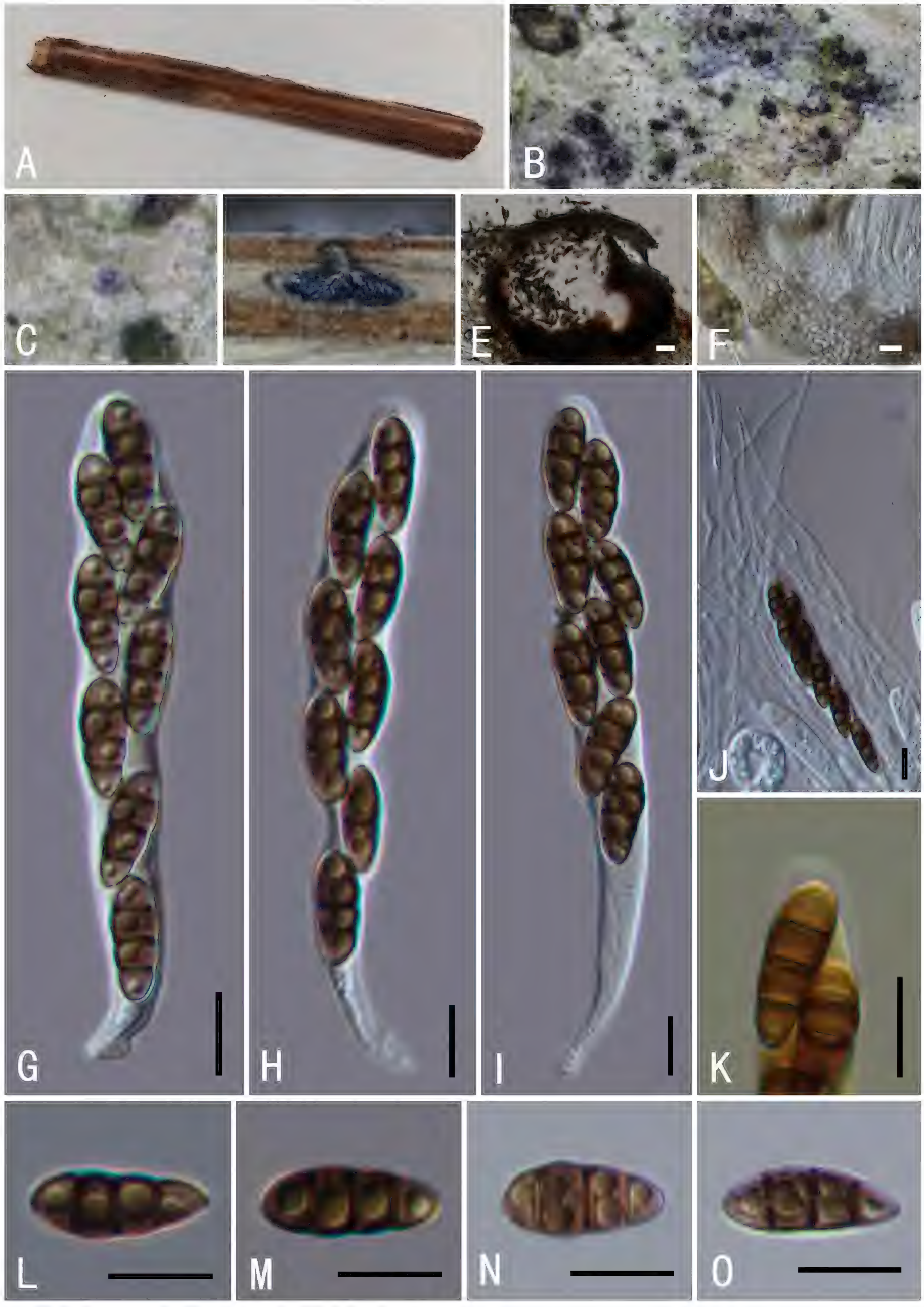

Figure 9. *Nigrograna trachycarpus* (GMB0499) **A** specimen **B, C** appearance of ascomata on substrate **D, E** longitudinal section of an ascoma **F** peridium **G–I** asci **J** pseudoparaphyses **K** J-ascus subapical ring in Melzer's **L–O** ascospores. Scale bars: 0.5 mm (**B–D**); 10 µm (**E–O**).

to dark brown cells of textura angularis. ***Hamathecium*** comprising numerous 1.4–2.2 µm diameter, filamentous, unbranched, anastomosing, septate pseudoparaphyses. ***Asci*** 86–126 × 11–13 µm (average = 99 × 12 µm, n = 25), 8-spored, bitunicate, with fissitunicate dehiscence occurring rarely, elliptical, shortly pedicellate, apically rounded, with an ocular chamber, with a J-subapical ring. ***Ascospores*** 15–17 × 5–7 µm (average = 16.3 × 6.1 µm, n = 40), hyaline to yellow brown, 2–3-septate, deeply constricted at second septum, tapering to each end, the widest point at second cell from apex, smooth-walled, distinctly guttulate, without a sheath or appendages. **Asexual morph**: undetermined.

Culture characteristics. After 4 weeks at 25 °C on PDA, colonies typically reach 2–2.5 cm in diameter. They present a circular shape with a dense and elevated centre, while appearing sparse and radiating at the margin. The colonies exhibit colours ranging from dark grey to pale olivaceous when viewed from above and from dark olivaceous to black on reverse.

Specimen examined. China, Guizhou Province, Guiyang Huaxi National Urban Wetland Park (26°2'2.34"N, 106°34'16.22"E), 1130 m elev., on decaying culms of *Trachycarpus* sp., 12 October 2022, Y.P Wu and H.M Hu, 2022 HXGY11 (GMB0499, holotype, GMBC0499, ex-type; KUN-HKAS 12984, isotype).

Other examined material. China, Guizhou Province, Qiannan Prefecture, Sandu Shui Autonomous County, Yao Man Mountain National Forest Park (25°93'18.76"N, 107°95'15.66"E), 540 m elev., on decaying bamboo culms of *Trachycarpus* sp.; 28 September 2022; Y.P. Wu, 2022YRS50 (GMB050; GMBC0505, living culture).

Notes. In the phylogenetic analysis, *Nigrograna trachycarpus* and *N. locuta-pollinis* F. Liu & L. Cai formed a monophyletic branch within the *Nigrograna* genus, with a bootstrap support value of 31% (Fig. 3). However, this relationship remained consistent in repeated phylogenetic analyses. Sequences generated from the cultures of *N. trachycarpus* are similar to sharing an ITS similarity of 70.7% (with 57/488 gaps) and a *tef*1 similarity of 89.8% (with 0/481 gaps). Morphologically, *N. trachycarpus* can be distinguished by its larger ascospores, measuring 16.3 × 6.1 µm, in contrast to *N. schinifolium*'s ascospores, 11.6 × 3.3 µm. Morphologically, it is close to *N. impatientis* J.F. Zhang, J.K. Liu & Z.Y. Liu, but the latter typically has ascocarps in groups of 2–6 with ostiole necks penetrating the host surface together. Moreover, the *N. trachycarpus* a possesses longer asci (measuring 99 × 12 µm) and larger ascospores (measuring 16.3 × 6.1 µm) compared to *N. impatientis* (asci measuring 48 × 8, ascospores measuring 12 × 4.3 µm) (Zhang et al. 2020a).

Discussion

In this study, based on phylogenetic trees of combined ITS, LSU, SSU, *tef*1 and *rpb*2 sequences and morphology, we described and illustrated three new species of micro-fungi on dead woody litter, viz., *Neokalmusia karka* (Didymosphaeriaceae), *Nigrograna schinifolium* and *N. trachycarpus* (Nigrograna ceae) and records of three species of *Roussoella* (Roussoellaceae). Didymosphaeriaceae was introduced by Munk (1953) and is one of the most diverse families within the *Pleosporales*, with a total of 33 genera (Thambugala et al. 2015; Haridas et al. 2020). We included all of these *Didymosphaeriaceae* genera in our phylogenetic analysis. We used a dataset that combines ITS, LSU, SSU, *tef*1

and *rpb*2 genes for this purpose. *Neokalmusia* formed a well-supported monophyletic clade within *Didymosphaeriaceae*, while the newly-discovered species, *N. karka*, exhibited a distinct separation from other known *Neokalmusia* species, supported by strong phylogenetic values.

Nigrograna, which is the only genus within *Nigrograna*ceae, is globally distributed and ecologically diverse. Amongst its species, *N. mackinnonii* is the most widely distributed species, mainly found in deciduous forests in Canada and northern USA. *Nigrograna bergmaniae* is mainly distributed in Europe, while *N. novae-zelandiae* was discovered in New Zealand. Approximately one-quarter of existing species live as saprotrophs on the bark or corticated twigs of various hardwoods (Phukhamsakda et al. 2018; Jayasiri et al. 2019). *Nigrograna schinifolium* was collected from rotten wood, while *N. trachycarpus* was obtained from decaying culms. Notably, several *Nigrograna* species have been established in recent studies without strong bootstrap value support. This finding suggests that these two species, *N. schinifolium* and *N. trachycarpus*, belong to the genus *Nigrograna* with strong evidence supporting this classification.

This study unveils valuable insights about saprophytic fungi, shedding light on their distribution and diversity within the Guizhou Region. It also identified three new species, which are important for the study of fungal taxonomy and further enriches our understanding of these microscopic organisms. Moreover, the study highlights the ongoing instability within the existing taxonomic system, emphasising the necessity for addressing these taxonomic challenges through processes such as re-collection, confirmation and sequencing of samples.

Additional information

Conflict of interest

The authors have declared that no competing interests exist.

Ethical statement

No ethical statement was reported.

Funding

This research was supported by National Natural Science Foundation of China (31960005, 32000009 and 32170019); Science and Technology Department Foundation of Guizhou Province ([2018]2322); Qianhe Talents, Science and Technology Department of Guizhou Province ([2015]4029); Guizhou Provincial Education Department Scientific Research Project for Higher Education Institutions ([2022]064); National Natural Science Foundation of China Karst Centre Project U1812403-4-4.

Author contributions

Conceptualization, Jichuan Kang, Qirui Li, Xiangchun Shen; investigation, Hongmin Hu, Youpeng Wu, Qingde Long; morpho-logical examinations, molecular sequencing, and phylogenetic analyses, Xu Zhang, Sihan Long and Youpeng Wu; specimen identification, Hongmin Hu and Qirui Li; writing—original draft preparation, Hongmin Hu, Minghui He; writing—review and editing, Nalin N. Wijayawardene, Zebin Meng; supervision, Qirui Li. All authors have read and agreed to the published version of the manuscript.

Author ORCIDs

Hongmin Hu https://orcid.org/0000-0003-3894-3269
Sihan Long https://orcid.org/0000-0002-8346-3646
Nalin N. Wijayawardene https://orcid.org/0000-0003-0522-5498

Data availability

All of the data that support the findings of this study are available in the main text.

References

Ahmed SA, Stevens DA, van de Sande WW, Meis JF, De Hoog GJ (2014a) *Roussoella percutanea*, a novel opportunistic pathogen causing subcutaneous mycoses. Medical Mycology 52(7): 689–698. https://doi.org/10.1093/mmy/myu035

Ahmed SA, Van De Sande W, Stevens DA, Fahal A, Van Diepeningen A, Menken S, De Hoog GS (2014b) Revision of agents of black-grain eumycetoma in the order Pleosporales. Persoonia 33(1): 141–154. https://doi.org/10.3767/003158514X684744

Ariyawansa HA, Camporesi E, Thambugala KM, Mapook A, Kang JC, Alias SA, Chukeatirote E, Thines M, McKenzie EH, Hyde KD (2014a) Confusion surrounding Didymosphaeria–phylogenetic and morphological evidence suggest Didymosphaeriaceae is not a distinct family. Phytotaxa 176. https://doi.org/10.11646/phytotaxa.176.1.12

Ariyawansa HA, Tanaka K, Thambugala KM, Phookamsak R, Tian Q, Camporesi E, Hyde KD (2014b) A molecular phylogenetic reappraisal of the Didymosphaeriaceae (= Montagnulaceae). Fungal Diversity 68(1): 69–104. https://doi.org/10.1007/s13225-014-0305-6

Ariyawansa HA, Hyde KD, Jayasiri SC, Buyck B, Chethana KT, Dai DQ, Dai YC, Daranagama DA, Jayawardena RS, Lücking R, Ghobad-Nejhad M, Niskanen T, Thambugala KM, Voigt K, Zhao RL, Li G-J, Doilom M, Boonmee S, Yang ZL, Cai Q, Cui Y-Y, Bahkali AH, Chen J, Cui BK, Chen JJ, Dayarathne MC, Dissanayake AJ, Ekanayaka AH, Hashimoto A, Hongsanan S, Jones EBG, Larsson E, Li WJ, Li Q-R, Liu JK, Luo ZL, Maharachchikumbura SSN, Mapook A, McKenzie EHC, Norphanphoun C, Konta S, Pang KL, Perera RH, Phookamsak R, Phukhamsakda C, Pinruan U, Randrianjohany E, Singtripop C, Tanaka K, Tian CM, Tibpromma S, Abdel-Wahab MA, Wanasinghe DN, Wijayawardene NN, Zhang J-F, Zhang H, Abdel-Aziz FA, Wedin M, Westberg M, Ammirati JF, Bulgakov TS, Lima DX, Callaghan TM, Callac P, Chang C-H, Coca LF, Dal-Forno M, Dollhofer V, Fliegerová K, Greiner K, Griffith GW, Ho H-M, Hofstetter V, Jeewon R, Kang JC, Wen T-C, Kirk PM, Kytövuori I, Lawrey JD, Xing J, Li H, Liu ZY, Liu XZ, Liimatainen K, Lumbsch HT, Matsumura M, Moncada B, Nuankaew S, Parnmen S, de Azevedo Santiago ALCM, Sommai S, Song Y, de Souza CAF, de Souza-Motta CM, Su HY, Suetrong S, Wang Y, Wei S-F, Wen TC, Yuan HS, Zhou LW, Réblová M, Fournier J, Camporesi E, Luangsa-ard JJ, Tasanathai K, Khonsanit A, Thanakitpipattana D, Somrithipol S, Diederich P, Millanes AM, Common RS, Stadler M, Yan JY, Li XH, Lee HW, Nguyen TTT, Lee HB, Battistin E, Marsico O, Vizzini A, Vila J, Ercole E, Eberhardt U, Simonini G, Wen H-A, Chen X-H, Miettinen O, Spirin V, Hernawati (2015) Fungal diversity notes 111–252–Taxonomic and phylogenetic contributions to fungal taxa. Fungal Diversity 75(1): 27–274. https://doi.org/10.1007/s13225-015-0346-5

Ariyawansa HA, Tsai I, Thambugala KM, Chuang WY, Lin SR, Hozzein WN, Cheewangkoon R (2020) Species diversity of Pleosporalean taxa associated with *Camellia sinensis* (L.) Kuntze in Taiwan. Scientific Reports 10(1): 1–20. https://doi.org/10.1038/s41598-020-69718-0

Barr, Margaret E (1987) Prodromus to Class Loculoascomycetes. Amherst. University of Massachusetts, Massachusetts.

Crous P, Cowan D, Maggs-Kölling G, Yilmaz N, Thangavel R, Wingfield M, Noordeloos ME, Dima B, Brandrud TE, Groenewald JZ (2021) Fungal planet description sheets: 1182–1283. Persoonia. Molecular Phylogeny and Evolution of Fungi 46: 313. https://doi.org/10.3767/persoonia.2021.46.11

Crous PW, Wingfield M, Guarro J, Hernández-Restrepo M, Sutton D, Acharya K, Barber P, Boekhout T, Dimitrov R, Groenewald JZ (2015b) Fungal planet description sheets: 320–370. Persoonia 34(1): 167–266. https://doi.org/10.3767/003158515X688433

Crous PW, Wingfield MJ, Guarro J, Cheewangkoon R, van der Bank M, Swart WJ, Stchigel AM, Cano-Lira JF, Roux J, Madrid H, Damm U, Wood AR, Shuttleworth LA, Hodges CS, Munster M, de Jesús Yáñez-Morales M, Zúñiga-Estrada L, Cruywagen EM, De Hoog GS, Silvera C, Najafzadeh J, Davison EM, Davison PJN, Barrett MD, Barrett RL, Manamgoda DS, Minnis AM, Kleczewski NM, Flory SL, Castlebury LA, Clay K, Hyde KD, Maússe-Sitoe SND, Chen S, Lechat C, Hairaud M, Lesage-Meessen L, Pawłowska J, Wilk M, Śliwińska-Wyrzychowska A, Mętrak M, Wrzosek M, Pavlic-Zupanc D, Maleme HM, Slippers B, Mac Cormack WP, Archuby DI, Grünwald NJ, Tellería MT, Dueñas M, Martín MP, Marincowitz S, de Beer ZW, Perez CA, Gené J, Marin-Felix Y, Groenewald JZ (2013) Fungal planet description sheets: 154–213. Persoonia 31(1): 188–296. https://doi.org/10.3767/003158513X675925

Crous PW, Wingfield MJ, Roux JJL, Richardson DM, Strasberg D, Shivas RG, Alvarado P, Edwards J, Moreno G, Sharma R, Sonawane MS, Tan YP, Altés A, Barasubiye T, Barnes CW, Blanchette RA, Boertmann D, Bogo A, Carlavilla JR, Cheewangkoon R, Daniel R, de Beer ZW, Yáñez-Morales M de Jesús, Duong TA, Fernández-Vicente J, Geering ADW, Guest DI, Held BW, Heykoop M, Hubka V, Ismail AM, Kajale SC, Khemmuk W, Kolařík M, Kurli R, Lebeuf R, Lévesque CA, Lombard L, Magista D, Manjón JL, Marincowitz S, Mohedano JM, Nováková A, Oberlies NH, Otto EC, Paguigan ND, Pascoe IG, Pérez-Butrón JL, Perrone G, Rahi P, Raja HA, Rintoul T, Sanhueza RMV, Scarlett K, Shouche YS, Shuttleworth LA, Taylor PWJ, Thorn RG, Vawdrey LL, Solano-Vidal R, Voitk A, Wong PTW, Wood AR, Zamora JC, Groenewald JZ (2015a) Fungal planet description sheets: 371–399. Persoonia 35: 264–327. https://doi.org/10.3767/003158515X690269

Crous PW, Wingfield MJ, Schumacher R, Summerell BA, Giraldo A, Gené J, Guarro J, Wanasinghe D, Hyde KD, Groenewald JZ (2014) Fungal planet description sheets: 281–319. Persoonia 33(1): 212–289. https://doi.org/10.3767/003158514X685680

Dai DQ, Phookamsak R, Wijayawardene NN, Li WJ, Bhat DJ, Xu JC, Taylor JE, Hyde KD, Chukeatirote EJ (2017) Bambusicolous fungi. Fungal Diversity 82(1): 1–105. https://doi.org/10.1007/s13225-016-0367-8

De Gruyter J, Woudenberg JHC, Aveskamp MM, Verkley GJM, Groenewald JZ, Crous PW (2012) Rediposition of phoma-like anamorphs in Pleosporales. Studies in Mycology 75: 1–36. https://doi.org/10.3114/sim0004

Dissanayake LS, Wijayawardene NN, Samarakoon MC, Hyde KD, Kang JC (2021) The taxonomy and phylogeny of *Austropleospora ochracea* sp. nov. (Didymosphaeriaceae) from Guizhou, China. Phytotaxa 491(3): 217–229. https://doi.org/10.11646/phytotaxa.491.3.2

Dong W, Wang B, Hyde KD, McKenzie EH, Raja HA, Tanaka K, Abdel-Wahab MA, Abdel-Aziz FA, Doilom M, Zhang H (2020) Freshwater Dothideomycetes. Fungal Diversity 105(1): 319–575. https://doi.org/10.1007/s13225-020-00463-5

Feng Y, Zhang SN, Liu ZY (2019) *Trematеia murispora* sp. nov. (Didymosphaeriaceae, Pleosporales) from Guizhou, China. Phytotaxa 416: 79–87. https://doi.org/10.11646/phytotaxa.416.1.10

Flakus A, Etayo J, Miadlikowska J, Lutzoni F, Kukwa M, Matura N, Rodriguez-Flakus P (2019) Biodiversity assessment of ascomycetes inhabiting Lobariella lichens in Andean cloud forests led to one new family, three new genera and 13 new species of lichenicolous fungi. Plant and Fungal Systematics. 64(2): 283–344. https://doi.org/10.2478/pfs-2019-0022

Gonçalves MFM, Vicente TFL, Esteves AC, Alves A (2019) Neptunomyces aureus gen. et sp. nov. (Didymosphaeriaceae, Pleosporales) isolated from algae in Ria de Aveiro, Portugal. MycoKeys 60: 31–44. https://doi.org/10.3897/mycokeys.60.37931

Hall TA (1999) BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. In Proceedings of the Nucleic acids symposium series. Nucleic acids symposium series, 95–98. https://doi.org/10.1021/bk-1999-0734.ch008

Haridas S, Albert R, Binder M, Bloem J, LaButti K, Salamov A, Andreopoulos B, Baker S, Barry K, Grigoriev IV (2020) 101 Dothideomycetes genomes: A test case for predicting lifestyles and emergence of pathogens. Studies in Mycology 96: 141–153. https://doi.org/10.1016/j.simyco.2020.01.003

Hongsanan S, Hyde KD, Phookamsak R, Wanasinghe DN, McKenzie EHC, Sarma VV, Boonmee S, Lücking R, Bhat DJ, Liu NG, Tennakoon DS, Pem D, Karunarathna A, Jiang SH, Jones EBG, Phillips AJL, Manawasinghe IS, Tibpromma S, Jayasiri SC, Sandamali DS, Jayawardena RS, Wijayawardene NN, Ekanayaka AH, Jeewon R, Lu YZ, Dissanayake AJ, Zeng XY, Luo ZL, Tian Q, Phukhamsakda C, Thambugala KM, Dai DQ, Chethana KWT, Samarakoon MC, Ertz D, Bao DF, Doilom M, Liu JK, PérezOrtega S, Suija A, Senwanna C, Wijesinghe SN, Konta S, Niranjan M, Zhang SN, Ariyawansa HA, Jiang HB, Zhang JF, Norphanphoun C, de Silva NI, Thiyagaraja V, Zhang H, Bezerra JDP, Miranda-González R, Aptroot A, Kashiwadani H, Harishchandra D, Sérusiaux E, Aluthmuhandiram JVS, Abeywickrama PD, Devadatha B, Wu HX, Moon KH, Gueidan C, Schumm F, Bundhun D, Mapook A, Monkai J, Chomnunti P, Suetrong S, Chaiwan N, Dayarathne MC, Yang J, Rathnayaka AR, Bhunjun CS, Xu JC, Zheng JS, Liu G, Feng Y, Xie N (2020) Refined families of Dothideomycetes: Dothideomycetidae and Pleosporomycetidae. Mycosphere 11(1): 1553–2107. https://doi.org/10.5943/mycosphere/11/1/13

Hyde KD, Dong Y, Phookamsak R, Jeewon R, Bhat DJ, Jones EG, Liu NG, Abeywickrama PD, Mapook A, Sheng J (2020) Fungal diversity notes 1151–1276: Taxonomic and phylogenetic contributions on genera and species of fungal taxa. Fungal Diversity 100(1): 5–277. https://doi.org/10.1007/s13225-020-00439-5

Hyde KD, Hongsanan S, Jeewon R, Bhat DJ, McKenzie EH, Jones EG, Phookamsak R, Ariyawansa HA, Boonmee S, Zhu L (2016) Fungal diversity notes 367–490: Taxonomic and phylogenetic contributions to fungal taxa. Fungal Diversity 80: 1–270. https://doi.org/10.1007/s13225-016-0373-x

Hyde KD, Norphanphoun C, Abreu VP, Bazzicalupo A, Thilini Chethana K, Clericuzio M, Dayarathne MC, Dissanayake AJ, Ekanayaka AH, He MQ, Hongsanan S, Huang S-K, Jayasiri SC, Jayawardena RS, Karunarathna A, Konta S, Kušan I, Lee H, Li J, Lin C-G, Liu N-G, Lu Y-Z, Luo Z-L, Manawasinghe IS, Mapook A, Perera RH, Phookamsak R, Phukhamsakda C, Siedlecki I, Soares AM, Tennakoon DS, Tian Q, Tibpromma S, Wanasinghe DN, Xiao Y-P, Yang J, Zeng X-Y, Abdel-Aziz FA, Li W-J, Senanayake IC,

Shang Q-J, Daranagama DA, de Silva NI, Thambugala KM, Abdel-Wahab MA, Bahkali AH, Berbee ML, Boonmee S, Bhat DJ, Bulgakov TS, Buyck B, Camporesi E, Castañeda-Ruiz RF, Chomnunti P, Doilom M, Dovana F, Gibertoni TB, Jadan M, Jeewon R, Jones EBG, Kang J-C, Karunarathna SC, Lim YW, Liu J-K, Liu Z-Y, Plautz Jr HL, Lumyong S, Maharachchikumbura SSN, Matočec N, McKenzie EHC, Mešić A, Miller D, Pawłowska J, Pereira OL, Promputtha I, Romero AI, Ryvarden L, Su H-Y, Suetrong S, Tkalčec Z, Vizzini A, Wen T-C, Wisitrassameewong K, Wrzosek M, Xu J-C, Zhao Q, Zhao R-L, Mortimer PE (2017) Fungal diversity notes 603–708: Taxonomic and phylogenetic notes on genera and species. Fungal Diversity 87(1): 1–235. https://doi.org/10.1007/s13225-017-0391-3

Jaklitsch W, Voglmayr H (2016) Hidden diversity in Thyridaria and a new circumscription of the Thyridariaceae. Studies in Mycology 85(1): 35–64. https://doi.org/10.1016/j.simyco.2016.09.002

Jayasiri S, Hyde K, Jones E, McKenzie E, Jeewon R, Phillips A, Bhat D, Wanasinghe D, Liu J, Karunarathna SC (2019) Diversity, morphology and molecular phylogeny of Dothideomycetes on decaying wild seed pods and fruits. Mycosphere 10(1): 1–186. https://doi.org/10.5943/mycosphere/10/1/1

Jiang HB, Hyde KD, Jayawardena RS, Doilom M, Xu J, Phookamsak R (2019) Taxonomic and phylogenetic characterizations reveal two new species and two new records of *Roussoella* (Roussoellaceae, Pleosporales) from Yunnan, China. Mycological Progress 18(4): 577–591. https://doi.org/10.1007/s11557-019-01471-9

Karunarathna A, Phookamsak R, Jayawardena RS, Cheewangkoon R, Hyde KD, Kuo CH (2019) The holomorph of *Neoroussoella alishanense* sp. nov. (Roussoellaceae, Pleosporales) on *Pennisetum purpureum* (Poaceae). Phytotaxa 406(4): 218–236. https://doi.org/10.11646/phytotaxa.406.4.1

Katoh K, Standley DM (2013) MAFFT multiple sequence alignment software version 7: Improvements in performance and usability. Molecular Biology and Evolution 30(4): 772–780. https://doi.org/10.1093/molbev/mst010

Kolařík M (2018) New taxonomic combinations in endophytic representatives of the genus Nigrograna. Czech Mycology 70(2): 123–126. https://doi.org/10.33585/cmy.70202

Kolařík M, Spakowicz DJ, Gazis R, Shaw J, Kubátová A, Nováková A, Chudíčková M, Forcina GC, Kang KW, Narváez-Trujillo A (2017) *Biatriospora* (Ascomycota: Pleosporales) is an ecologically diverse genus including facultative marine fungi and endophytes with biotechnological potential. Plant Systematics and Evolution 303(1): 35–50. https://doi.org/10.1007/s00606-016-1350-2

Kruys S, Eriksson OE, Wedin M (2006) Phylogenetic relationships of coprophilous Pleosporales (Dothideomycetes, Ascomycota), and the classification of some bitunicate taxa of unknown position. Mycological Research 110(5): 527–536. https://doi.org/10.1016/j.mycres.2006.03.002

Liang H, Gagné JD, Faye A, Rodrigue D, Brisson J (2020) Ground tire rubber (GTR) surface modification using thiol-ene click reaction: Polystyrene grafting to modify a GTR/polystyrene (PS) blend. Progress in Rubber, Plastics and Recycling Technology 36(2): 81–101. https://doi.org/10.1177/1477760619895016

Liu JK, Phookamsak R, Dai DQ, Tanaka K, Jones BG, Xu J-C, Chukeatirote E, Hyde KD (2014) Roussoellaceae, a new pleosporalean family to accommodate the genera *Neoroussoella* gen. nov., *Roussoella* and *Roussoellopsis*. Phytotaxa 181(1): 1–33. https://doi.org/10.11646/phytotaxa.181.1.1

Long QD, Liu LL, Zhang X, Wen TC, Kang JC, Hyde KD, Shen XC, Li QR (2019) Contributions to species of Xylariales in China-1. Durotheca species. Mycological Progress 18(3): 495–510. https://doi.org/10.1007/s11557-018-1458-6

Mapook A, Hyde KD, McKenzie EH, Jones EG, Bhat DJ, Jeewon R, Stadler M, Samarakoon MC, Malaithong M, Purahong W (2020) Taxonomic and phylogenetic contributions to fungi associated with the invasive weed *Chromolaena odorata* (Siam weed). Fungal Diversity 101(1): 1–175. https://doi.org/10.1007/s13225-020-00444-8

Munk A (1953) The system of the pyrenomycetes. A contribution to a natural classification of the group Sphaeriales *sensu* Lindau. Dansk Bot Ark 15: 1–163.

Phukhamsakda C, Bhat DJ, Hongsanan S, Xu JC, Stadler M, Hyde KD (2018) Two novel species of *Neoaquastroma* (Parabambusicolaceae, Pleosporales) with their phoma-like asexual morphs. MycoKeys 34: 47–62. https://doi.org/10.3897/mycokeys.34.25124

Poli A, Bovio E, Ranieri L, Varese GC, Prigione V (2020) News from the sea: A new genus and seven new species in the Pleosporalean families Roussoellaceae and Thyridariaceae. Diversity 12(4): 144. https://doi.org/10.3390/d12040144

Posada D, Crandall KA (1998) MODELTEST: Testing the model of DNA substitution. Bioinformatics 14(9): 817–818. https://doi.org/10.1093/bioinformatics/14.9.817

Ramesh C, (2003) Loculoascomycetes from India. Frontiers of Fungal Diversity in India: Prof. Kamal Festschrif, 457–479.

Rogers JD, Ju YM (1996) *Entoleuca mammata* comb. nov. for *Hypoxylon mammatum* and the genus *Entoleuca*. Mycotaxon 59: 441–448. https://doi.org/10.1007/s11557-017-1311-3

Ronquist F, Teslenko M, Van Der Mark P, Ayres DL, Darling A, Höhna S, Larget B, Liu L, Suchard MA, Huelsenbeck JP (2012) MrBayes 3.2: Efficient Bayesian phylogenetic inference and model choice across a large model space. Systematic Biology 61(3): 539–542. https://doi.org/10.1093/sysbio/sys029

Saccardo PA (1888) Sylloge Hymenomyceteae, Vol. VI. Polyporeae, Hydneae, Thelephoreae, Clavarieae, Tremellineae. Sylloge Fungorum 6: 1–928.

Schoch CL, Crous PW, Groenewald JZ, Boehm EW, Burgess TI, de Gruyter J, de Hoog GS, Dixon LJ, Grube M, Gueidan C, Harada Y, Hatakeyama S, Hirayama K, Hosoya T, Huhndorf SM, Hyde KD, Jones EB, Kohlmeyer J, Kruys A, Li YM, Lücking R, Lumbsch HT, Marvanová L, Mbatchou JS, McVay AH, Miller AN, Mugambi GK, Muggia L, Nelsen MP, Nelson P, Owensby CA, Phillips AJ, Phongpaichit S, Pointing SB, Pujade-Renaud V, Raja HA, Plata ER, Robbertse B, Ruibal C, Sakayaroj J, Sano T, Selbmann L, Shearer CA, Shirouzu T, Slippers B, Suetrong S, Tanaka K, Volkmann-Kohlmeyer B, Wingfield MJ, Wood AR, Woudenberg JH, Yonezawa H, Zhang Y, Spatafora JW (2009) A class-wide phylogenetic assessment of Dothideomycetes. Stud Mycol 64: 1-15S10. https://doi.org/10.3114/sim.2009.64.01.

Senwanna C, Hyde KD, Phookamsak R, Jones EG, Cheewangkoon R (2018) *Coryneum-heveanum* sp. nov. (Coryneaceae, Diaporthales) on twigs of Para rubber in Thailand. MycoKeys 75: 75–90. https://doi.org/10.3897/mycokeys.43.29365

Seong KL, Kallol D, Soo MH, Sang JS, Seung YL, Hee YJ (2023) Morphological and Phylogenetic Analyses Reveal a New Species of Genus Monochaetia Belonging to the Family Sporocadaceae in Korea. Mycobiology 51(2): 87–93. https://doi.org/10.1080/12298093.2022.2038843

Tanaka K, Hirayama K, Yonezawa H, Hatakeyama S, Harada Y, Sano T, Shirouzu T, Hosoya TJ (2009) Molecular taxonomy of bambusicolous fungi: Tetraplosphaeriaceae, a new pleosporalean family with Tetraploa-like anamorphs. Studies in Mycology 64: 175–209. https://doi.org/10.3114/sim.2009.64.10

Tanaka K, Hirayama K, Yonezawa H, Sato G, Toriyabe A, Kudo H, Hashimoto A, Matsumura M, Harada Y, Hosoya T (2015) Revision of the Massarineae (Pleosporales, Dothideomycetes). Studies in Mycology 82(1): 75–136. https://doi.org/10.1016/j.simyco.2015.10.002

Thambugala K, Wanasinghe D, Phillips A, Camporesi E, Bulgakov T, Phukhamsakda C, Ariyawansa H, Goonasekara I, Phookamsak R, Hyde KD (2017) Mycosphere notes 1–50: Grass (Poaceae) inhabiting Dothideomycetes. Mycosphere 8(4): 697–796. https://doi.org/10.5943/mycosphere/8/4/13

Thambugala KM, Hyde KD, Tanaka K, Tian Q, Wanasinghe DN, Ariyawansa HA, Jayasiri SC, Boonmee S, Camporesi E, Liu ZY (2015) Towards a natural classification and backbone tree for Lophiostomataceae, Floricolaceae, and Amorosiaceae fam. nov. Fungal Diversity 74(1): 199–266. https://doi.org/10.1007/s13225-015-0348-3

Tibpromma S, Hyde KD, Jeewon R, Maharachchikumbura SS, Liu JK, Bhat DJ, Jones EG, McKenzie EH, Camporesi E, Bulgakov TS, Doilom M, de Azevedo Santiago ALCM, Das K, Manimohan P, Gibertoni TB, Lim YW, Ekanayaka AH, Thongbai B, Lee HB, Yang J-B, Kirk PM, Sysouphanthong P, Singh SK, Boonmee S, Dong W, Raj KNA, Latha KPD, Phookamsak R, Phukhamsakda C, Konta S, Jayasiri SC, Norphanphoun C, Tennakoon DS, Li J, Dayarathne MC, Perera RH, Xiao Y, Wanasinghe DN, Senanayake IC, Goonasekara ID, de Silva NI, Mapook A, Jayawardena RS, Dissanayake AJ, Manawasinghe IS, Chethana KWT, Luo Z-L, Hapuarachchi KK, Baghela A, Soares AM, Vizzini A, Meiras-Ottoni A, Mešić A, Dutta AK, de Souza CAF, Richter C, Lin C-G, Chakrabarty D, Daranagama DA, Lima DX, Chakraborty D, Ercole E, Wu F, Simonini G, Vasquez G, da Silva GA, Plautz Jr HL, Ariyawansa HA, Lee H, Kušan I, Song J, Sun J, Karmakar J, Hu K, Semwal KC, Thambugala KM, Voigt K, Acharya K, Rajeshkumar KC, Ryvarden L, Jadan M, Hosen MI, Mikšík M, Samarakoon MC, Wijayawardene NN, Kim NK, Matočec N, Singh PN, Tian Q, Bhatt RP, de Oliveira RJV, Tulloss RE, Aamir S, Kaewchai S, Marathe SD, Khan S, Hongsanan S, Adhikari S, Mehmood T, Bandyopadhyay TK, Svetasheva TY, Nguyen TTT, Antonín V, Li W-J, Wang Y, Indoliya Y, Tkalčec Z, Elgorban AM, Bahkali AH, Tang AMC, Su H-Y, Zhang H, Promputtha I, Luangsa-ard J, Xu J, Yan J, Ji-Chuan K, Stadler M, Mortimer PE, Chomnunti P, Zhao Q, Phillips AJL, Nontachaiyapoom S, Wen T-C, Karunarathna SC (2017) Fungal diversity notes 491–602: Taxonomic and phylogenetic contributions to fungal taxa. Fungal Diversity 83(1): 1–261. https://doi.org/10.1007/s13225-017-0378-0

Tibpromma S, Hyde KD, McKenzie EH, Bhat DJ, Phillips AJ, Wanasinghe DN, Samarakoon MC, Jayawardena RS, Dissanayake AJ, Karunarathna SC (2018) Fungal diversity notes 840–928: Micro-fungi associated with Pandanaceae. Fungal Diversity 93(1): 1–160. https://doi.org/10.1007/s13225-018-0408-6

Valenzuela-Lopez N, Sutton DA, Cano-Lira JF, Paredes K, Wiederhold N, Guarro J, Stchigel AM (2017) Coelomycetous fungi in the clinical setting: Morphological convergence and cryptic diversity. Journal of Clinical Microbiology 55(2): 552–567. https://doi.org/10.1128/JCM.02221-16

Verkley G, Dukik K, Renfurm R, Göker M, Stielow JB (2014) Novel genera and species of coniothyrium-like fungi in Montagnulaceae (Ascomycota). Persoonia 32(1): 25–51. https://doi.org/10.3767/003158514X679191

Vu D, Groenewald M, De Vries M, Gehrmann T, Stielow B, Eberhardt U, Al-Hatmi A, Groenewald JZ, Cardinali G, Verkley GJ (2019) Large-scale generation and analysis of filamentous fungal DNA barcodes boosts coverage for kingdom fungi and reveals thresholds for fungal species and higher taxon delimitation. Studies in Mycology 92(1): 135–154. https://doi.org/10.1016/j.simyco.2018.05.001

Wanasinghe DN, Jones EB, Camporesi E, Dissanayake AJ, Kamolhan S, Mortimer PE, Xu J, Abd-Elsalam KA, Hyde KD (2016) Taxonomy and phylogeny of *Laburnicola* gen. nov. and *Paramassariosphaeria* gen. nov. (Didymosphaeriaceae, Massarineae, Pleosporales). Fungal Biology 120(11): 1354–1373. https://doi.org/10.1016/j.funbio.2016.06.006

Wanasinghe DN, Phukhamsakda C, Hyde KD, Jeewon R, Lee HB, Gareth Jones E, Tibpromma S, Tennakoon DS, Dissanayake AJ, Karunarathna SC (2018) Fungal diversity notes 709–839: Taxonomic and phylogenetic contributions to fungal taxa with an emphasis on fungi on Rosaceae. Fungal Diversity 89(1): 1–236. https://doi.org/10.1007/s13225-018-0395-7

Wijayawardene N, Hyde KD, Al-Ani L, Tedersoo L, Ags SF (2020) Outline of Fungi and fungi-like taxa. Mycosphere 11(1): 1160–1456. https://doi.org/10.5943/mycosphere/11/1/8

Wijayawardene NN, Hyde KD, Bhat DJ, Camporesi E, Schumacher RK, Chethana KT, Wikee S, Bahkali AH, Wang Y (2014) Camarosporium-like species are polyphyletic in Pleosporales; introducing *Paracamarosporium* and *Pseudocamarosporium* gen. nov. in Montagnulaceae. Cryptogamie. Mycologie 35(2): 177–198. https://doi.org/10.7872/crym.v35.iss2.2014.177

Wijayawardene NN, Hyde KD, Dai DQ, Sánchez-García M, Goto BT, Magurno F (2022) Outline of Fungi and fungus-like taxa–2021. Mycosphere 13(1): 53–453. https://doi.org/10.5943/mycosphere/13/1/2

Wijesinghe SN, Wanasinghe DN, Maharachchikumbura SS, Wang Y, Al-Sadi AM, Hyde KD (2020) *Bimuria omanensis* sp. nov. (Didymosphaeriaceae, Pleosporales) from Oman. Phytotaxa 449(2): 97–108. https://doi.org/10.11646/phytotaxa.449.2.1

Zhang JF, Liu JK, Thambugala KM, Yang J, Meng ZH, Liu ZY (2020a) Two new species and a new record of *Nigrograna* (Nigrograna ceae, Pleosporales) from China and Thailand. Mycological Progress 19(11): 1365–1375. https://doi.org/10.1007/s11557-020-01633-0

Zhang JY, Phookamsak R, Boonmee S, Hyde KD, Dai DQ, Lu YZ (2020b) *Roussoella guttulata* (Roussoellaceae, Pleosporales), a novel bambusicolous ascomycete from Thailand. Phytotaxa 471: 221–233. https://doi.org/10.11646/phytotaxa.471.3.4

Zhang Y, Crous PW, Hyde SK (2012) Pleosporales. Fungal Diversity 53(1): 1–221. https://doi.org/10.1007/s13225-011-0117-x

Zhang Y, Fournier J, Phookamsak R, Bahkali AH, Hyde KD (2013) Halotthiaceae fam. nov. (Pleosporales) accommodates the new genus *Phaeoseptum* and several other aquatic genera. Mycologia 105(3): 603–609. https://doi.org/10.3852/11-286

Zhao Y, Zhang Z, Cai L, Peng W, Liu FJ (2018) Four new filamentous fungal species from newly-collected and hive-stored bee pollen. Mycosphere 9(6): 1089–1116. https://doi.org/10.5943/mycosphere/9/6/3